WEST

Generate Collection Print

L4: Entry 7 of 8 5,602,110 File: USPT Feb 11, 1997

DOCUMENT-IDENTIFIER: US 5602110 A
TITLE: Method and composition for treating cystic fibrosis

Detailed Description Text (2):
The present invention includes a method and composition for treating cystic fibrosis. The method comprises administering to a patient, such as a human, suffering from cystic fibrosis a treatment comprising, in combination, an amount of a first component and an amount of a second component, said first component being an inhibitor which is specific for a cGMP-inhibited type III cAMP phosphodiesterase (sometimes referred to herein as "Type III phosphodiesterase inhibitors"), said second component being an adenylate cyclase activator, the amount of the first component and the amount of the second component, in combination, being a therapeutically effective treatment amount. As used herein in the claims, the terms or phrases "an inhibitor which is specific for a cGMP-inhibited type III cAMP phosphodiesterase", "adenylate cyclase activator", ".beta.-adrenergic receptor agonist", "cAMP", "a cAMP analog which activates protein kinase A", and the members of these groups or classes, are defined to include their pharmaceutically acceptable derivations including pharmaceutically acceptable salts such as metal ion salts, for example alkali metal salts, and their complexes, such as when the free base complexes with a pharmaceutically acceptable acid, for example albuterol sulfate, isoproterenol hydrochloride, pirbuterol acetate, epinephrine bitartrate, isoproterenol sulfate, and terbutaline sulfate.

Detailed Description Text (40):
The effects of milrinone and isoproterenol were enhanced by the addition of Sp-adenosine 3':5'-cyclic phosphorothioate ("Sp-cAMPS"), an analog of cAMP which is known to activate protein kinase A. As shown in FIG. 2, the increased chloride efflux. Sp-cAMPS stimulated by isoproterenol and milrinone was increased by Sp-cAMPS The stereoisomer of Sp-cAMPS, Rp-cAMPS, an inhibitor of protein kinase A, abolished the increase in chloride efflux. Sp-cAMPS alone had no effect on chloride efflux. It is believed that cAMP, and other cAMP analogs which activate protein kinase A, particularly those which have a higher affinity for protein kinase A than cAMP's affinity for protein kinase A, will act synergistically similar to Sp-cAMPS.

WEST

Generate Collection | Print

5,792,752

L3: Entry 15 of 18 File: USPT Aug 11, 1998

DOCUMENT-IDENTIFIER: US 5792752 A
TITLE: 8-chloro camp and related camp compounds as antineoplastic agents

Detailed Description Text (63):
A variety of cAMP analogues, modified at either the C-6 or C-8 positions of the adenine moiety at various concentrations, were tested for their growth inhibitory effect on leukemic cell lines, as shown in Table 11. Among the C-8 analogues (site 1-selective) tested, 8-Cl-cAMP exhibited the most potency, demonstrating 50% growth inhibition at 5-20 micromol/L concentrations (IC.sub.50) in all four leukemic cell lines. 8-Br-, 8-methylthio, and 8-methylamino-cAMP were 5 to 20 times less potent than 8-Cl-cAMP. N.sup.6 -benzyl-cAMP was the most potent of the C-6 analogues (site 2-selective) tested with IC.sub.50 values of 10 to 30 micromol/L. N.sup.6 -benzoyl-cAMP, which is structurally similar to N.sup.6 -benzyl-cAMP, exhibited IC50 values of 40 to 50 micromol/L. DBcAMP, the analogue most commonly used in previous studies, exhibited the least potency, the IC.sub.50 values of 500 to 1000 micromol/L, and in Molt-4, the 50% growth inhibition could not be obtained. Growth inhibition by the site-selective cAMP analogues was not due to cell killing; the cells were 80% to 90% viable as determined by exclusion of trypan blue dye.

Detailed Description Text (81):
In addition to the cAMP analogue or combination of cAMP analogues and pharmaceutically acceptable salts thereof of the present invention, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferably, the preparations, particularly those which can be administered orally and which can be used for the preferred type of administration, such as tablets, dragees, and capsules, and also preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection or orally, contain from about 0.1 to 99 percent, preferably from about 25 to 85 percent by weight of active ingredient, together with the excipient.

Other Reference Publication (22):
Cho-Chung et al. (III), "Role of Site-Selective cAMP Analogs in the Control and Reversal of Malignancy," Pharmac. Ther., 50(1), 1-33 (1991).

CLAIMS:

1. A method of inhibiting the neoplastic growth of cancer cells comprising contacting said cells with a neoplastic growth inhibiting-effective amount of a cAMP derivative modified at the C-8, C-6, or C-6 and C-8 positions, wherein said C-6 substituent is selected from the group consisting of monobenzyl, monoethoxycarbonyl, monobenzoyl, monophenylcarbamoyl, monobutyryl, monobutyl, monophenyl, and diethyl or N-piperidino, and wherein said C-8 substituent is selected from the group consisting of halogen, methylthio, p-chlorophenylthio, .beta.-hydroxyethylamino and methylamino, and pharmaceutically acceptable salts of said C-6. C-8 and C-8 cAMP derivatives, and wherein said C-8 or C-6 and C-8 modified derivative selectively binds to type II cAMP dependent protein kinase at site 1 and said C-6 or C-6 and C-8 modified derivative selectively binds to types I and II cAMP dependent protein kinases at site 2.

4. The method of claim 3, wherein said growth-inhibiting cAMP derivative is combined

with a pharmaceutical carrier.

21. A pharmaceutical composition consisting essentially of a first derivative of cAMP modified at the C-8 position, a second derivative of cAMP modified at the C-6 position, wherein said derivative selectively binds to type II cAMP dependent protein kinase at site 1, and wherein said second derivative selectively binds to type I and type II cAMP dependent protein kinase at site 2, and a pharmaceutically acceptable carrier.

WEST

Generate Collection Print

5,843,916

L3: Entry 14 of 18 File: USPT Dec 1, 1998

DOCUMENT-IDENTIFIER: US 5843916 A
TITLE: Cyclic amp analogues, individually and in pairs, to inhibit neoplastic cell growth

Abstract Text (1):
A method of inhibiting the proliferation of cells, particularly cancerous cells, by contacting the cells with a phosphorothioate derivative of a cAMP modified at either or both the C-6 and C-8 positions of the adenine moiety, and pharmaceutical compositions therefor.

Brief Summary Text (25):
It is another object of the present invention to provide compositions, in particular pharmaceutical compositions, comprising such improved derivatives of cAMP analogues.

Detailed Description Text (2):
The present invention involves a method of inhibiting the proliferation of cells by contacting the cells with an effective amount of a compound selected from the group consisting of phosphorothioate derivatives of cAMP modified at either or both the C-6 and C-8 positions of the adenine moiety and pharmaceutically acceptable salts thereof. Of particular interest are those phosphorothioate derivatives of 8-halo-cAMP, preferably of 8-Cl-cAMP and 8-Br-cAMP. The most preferred phosphorothioate derivatives are those of Rp-8-Cl-cAMPS and Sp-8-Br-cAMPS, particularly Rp-8-Cl-cAMPS.

Detailed Description Text (3):
The present invention also includes such a method wherein the cells are also contacted with an additional compound selected from the group consisting of 8-Cl-cAMP, N.sup.6 -benzyl-cAMP, N.sup.6 -benzoyl-8-methylthio-cAMP, 8-methylthio-cAMP, N.sup.6 -benzoyl-cAMP, 8-bromo-cAMP, 8-iodo-cAMP, 8-p-chlorophenylthio-cAMP, 8-.beta.-hydroxyethylamino-cAMP, 8-methylamino-cAMP, 8-N,N-dimethylamino-cAMP, N.sup.6 -phenylcarbamoyl-cAMP, N.sup.6 -butyryl-cAMP, N.sup.6, O.sup.2 -dibutyryl-cAMP, N.sup.6 -phenyl-8-p-chlorophenylthio-cAMP, N.sup.6,N .sup.6 -diethyl-8-p-chlorophenylthio-cAMP, 6-piperidino-8-p-chlorophenylthio-cAMP, N.sup.6 -benzyl-8-benzylthio-cAMP, N.sup.6 -ethoxycarbonyl-cAMP, N.sup.6 -n-butyl-8-p-chlorophenylthio-cAMP, pharmaceutically acceptable salts thereof, and mixtures thereof, particularly 8-Cl-cAMP, N.sup.6 -benzyl-cAMP, and pharmaceutically acceptable salts thereof. The cells may be contacted with these compounds simultaneously, e.g., in a single pharmaceutical composition, or sequentially, e.g., in an alternating sequence.

Detailed Description Text (61):
A variety of cAMP analogues, modified at either the C-6 or C-8 positions of the adenine moiety at various concentrations, were tested for their growth inhibitory effect on leukemic cell lines, as shown in Table II. Among the C-8 analogues (site 1-selective) tested, 8-Cl-cAMP exhibited the most potency, demonstrating 50% growth inhibition at 5-20 .mu.M concentrations (IC.sub.50) in all four leukemic cell lines. 8-Br-, 8-methylthio, and 8-methylamino-cAMP were 5 to 20 times less potent than 8-Cl-cAMP. N.sup.6 -benzyl-cAMP was the most potent of the C-6 analogues (site 2-selective) test with IC.sub.50 values of 10 to 30 .mu.M N.sup.6 -benzoyl-cAMP, which is structurally similar to N.sup.6 -benzyl-cAMP, exhibited IC.sub.50 values of 40 to 50 .mu.M. DBcAMP, the analogue most commonly used in previous studies,

exhibited the least potency, with IC.sub.50 values of 500 to 1000 .mu.M, and in Molt-4, the 50% growth inhibition could not be obtained. Growth inhibition by the site-selective cAMP analogues was not due to cell killing; the cells were 80% to 90% viable as determined by exclusion of trypan blue dye.

Detailed Description Text (82):
This example describes the binding affinities of Rp- and Sp- analogues of cAMP. 8-chloro-adenosine 31:51-monophosphate (8-Cl-cAMP) was obtained from the National Cancer Institute, Division of Cancer Treatment, Bethesda, Md. 8-bromo-adenosine 3': 5'-monophosphate (8-Br-cAMP) was obtained from Sigma Chemical Company, St. Louis, Mo. Rp- (equatorial exocyclic sulfur substitution) and Sp- (axial exocyclic sulfur substitution) 8-chloro-adenosine 3': 5'-monophosphorothioate (cAMPS) and -cAMPS analogues were synthesized as described in O'Brian et al. (Biochemistry 21: 4371-4376 (1982)), Dostmann (Ph.D. dissertation, Universitat Bremen, Germany, 1987), and Genieser et al. (Tetrahedron Lett. 19: 2803-2804 (1988)).

Detailed Description Text (91):
The concentrations of cAMP analogues that inhibit 50% of cell proliferation (IC.sub.50) are shown in Table 16. The IC.sub.50 values of all of the analogues at day 6 were 10- to 12-fold lower than those at day 3, indicating that the sensitivity of cells to analogues increased with the length of time of treatment. The IC.sub.50 values of day 6 indicate that the Rp- and Sp-phosphorothioates of 8-Cl-cAMP were 10- and 20-fold less potent, respectively, than 8-Cl-cAMP. In contrast, Sp-8-Br-cAMPS, which exhibited the same IC.sub.50 value as that of Rp-8-Cl-cAMPS, was 6-fold more potent than 8-Br-cAMP.

Detailed Description Text (103):
Diastereomeric phosphorothioates of cAMP analogues have been shown to be potent inhibitors of the growth of human cancer cell lines. Rp-8-Cl-cAMPS and Sp-8-Br-cAMPS, both of which exhibit IC.sub.50 of 3 .mu.M in HL-60 leukemia cells, are the two most potent growth inhibitors among the phosphorothioates tested. The growth inhibitory effect of Rp-8-Cl-cAMPS was about 10-fold lower than that of 8-Cl-cAMP but 3-fold greater than that of Sp-8-Cl-cAMPS. The mechanism of action of Rp-8-Cl-cAMPS in growth inhibition appears to be similar to that of 8-Cl-cAMP. Rp-8-Cl-cAMPS did not bring about any change in cell cycle phase like 8-Cl-cAMP (Tortora et al., Blood 71: 230-233 (1988)). Rp-8-Cl-cAMPS enhanced its growth inhibitory effect when in combination with 8-Cl-cAMP and increased its cell differentiation effect when in combination with N.sup.6 -benzyl-cAMP like 8-Cl-cAMP in combination with N.sup.6 -benzyl-cAMP (Tortora et al., PNAS USA 86: 2849-2852 (1989)).

Detailed Description Text (109):
In addition to the cAMP analogue or combination of cAMP analogues and pharmaceutically acceptable salts thereof of the present invention, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferably, the preparations, particularly those which can be administered orally and which can be used for the preferred type of administration, such as tablets, dragees, and capsules, and also preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection or orally, contain from about 0.1 to 99 percent, preferably from about 25 to 85 percent by weight of active ingredient, together with the excipient.

Detailed Description Paragraph Table (14):
TABLE 14 Binding affinity of Sp- and Rp- and cyclophosphate analogues of cAMP for cAMP receptor proteins, RI and RII RI and RII are the regulatory subunits of cAMP-dependent protein kinase type I and type II, respectively. Site A (Site 2) and Site B (Site 1) are two distinct cAMP binding sites on cAMP receptor proteins (RI and RII): the affinities of analogues for Site A and Site B were determined by the measurement of kinetic constants (inhibition constant, K.sub.i), and the values are expressed as the relative affinity K.sub.i (cAMP)/K.sub.i (analogue), i.e., the ratio between the apparent inhibition constant for cAMP and the analogue, as described in Ogreid et al., Eur. J. Biochem. 150:219-227 (1985). RI RII Analogue Site A Site B Site A Site B

Sp-8-Cl-cAMPS 0.18 0.037 0.0023 2.4 Rp-8-Cl-cAMPS 0.0079 0.0042 0.00028 0.44 Sp-8-Br-cAMPS.sup.a 0.19 0.054 0.003 2.2 8-Cl-cAMPb 2.71 2.0 0.051 4.61 8-Br-cAMPC 1.30 1.0 0.11 6.8 .sup.a Dostmann et al., J. Biol. Chem., 265: 10484-10491 (1990). .sup.b Ally et al., Proc. Natl. Acad. Sci. USA. 85: 6319-6322 (1988). .sup.c Ogreid et al., Eur. J. Biochem. 181: 19-31 (1989).

Detailed Description Paragraph Table (16):
TABLE 16 Growth inhibition of HL-60 human leukemia cells by derivatives of cAMP analogues The IC.sub.50 values were determined from the dose- response curve experiments like those shown in FIG. 1 and represent an average value obtained for each analogue from two or more separate experiments carried out from 3 days or 6 days. Initial cell number, 1 .times. 10.sup.5 /dish. Typical numbers of untreated control cells at day 3 and day 6 were 265,000 and 1,011,840, respectively. 99% cell viability in treated and control cells. Growth inhibition (IC.sub.50) (.mu.M) Analogue Day 3 Day 6 Rp-8-Cl-cAMPS 16 3 Sp-8-Cl-cAMPS 100 8 Sp-8-Br-cAMPS 28 3 8-Br-cAMP 100 18 8-Cl-cAMP 5 0.4

Detailed Description Paragraph Table (18):
TABLE 18 Effect of phosphorothioate derivatives of cAMP analogues on the growth of LS-174T human colon carcinoma cells The percentage growth inhibition values shown at 10 and 50 .mu.M analogue concentrations were determined from the dose-response curve experiments like those shown in FIG. 1 and represent an average value obtained for each analogue from two or more separate experiments. Sp-6-SET- cPMPS: Sp-6-ethylthiopurine-3':5'-monophosphorothioate; Sp-2-Cl-cAMPS: Sp-2-Chloroadenosine-3':5'- monophosphorothioate. % Growth inhibition at 10 .mu.M 50 .mu.M Rp-8-Cl-cAMPS 48 70 Sp-8-Br-cAMPS 40 60 Sp-6-SET-cPMPS 25 50 Sp-8-Cl-cAMPS 20 40 Rp-cAMPS 15 35 Sp-2-Cl-cAMPS 10 25 Sp-cAMPS 7 15

Other Reference Publication (8):
Cho-chung et al.(II),"Role of Site-Selective cAMP Analogs in the Control and Reversal of Malignancy,"Pharmac. Ther., 50, 1-33 (1991).

CLAIMS:

1. A method of inhibiting neoplastic cellular growth, which method comprises contacting neoplastic cells with an effective neoplastic cellular growth inhibiting amount of a compound selected from the group consisting of phosphorothioate derivatives of cAMP analogues, wherein said cAMP analogues have been modified at either or both the N.sup.6 or C-8 positions of the adenine moiety, wherein said N.sup.6 - or C-6 substitution is selected from the group consisting of monobenzyl, monoethoxycarbonyl, monobenzoyl, monophenylcarbamoyl, monobutyryl, monophenyl, and diethyl or N-piperidino, and wherein said C-8 substituent is selected from the group consisting of halogen, methylthio, p-chlorophenylthio, .beta.-hydroxyethylamino and methylamino, and pharmaceutically acceptable salts thereof.

7. The method of claim 1, which further comprises contacting said cells with an effective amount of an additional compound selected from the group consisting of 8-Cl-cAMP, N.sup.6 -benzyl-cAMP, N.sup.6 -benzoyl-8-methylthio-cAMP, 8-methylthio-cAMP, N.sup.6 -benzoyl-cAMP, 8-bromo-cAMP, 8-iodo-cAMP, 8-p-chlorophenyl-thio-cAMP, 8-.beta.-hydroxyethylamino-cAMP, 8-methylamino-cAMP, 8-N,N-dimethylamino-cAMP, N.sup.6 -phenylcarbamoyl-cAMP, N.sup.6 -butyryl-cAMP, N.sup.6 -phenyl-8-p-chlorophenylthio-cAMP, N.sup.6,N.sup.6 -diethyl-8-p-chlorophenylthio-cAMP, 6-piperidino-8-p-chlorophenylthio-cAMP, N.sup.6 -benzyl-8-benzylthio-cAMP, N.sup.6 -ethoxycarbonyl-cAMP, N.sup.6 -n-butyl-8-p-chlorophenylthio-cAMP, pharmaceutically acceptable salts thereof, and mixtures thereof.

8. The method of claim 7, wherein said additional compound is selected from the group consisting of 8-Cl-cAMP and pharmaceutically acceptable salts thereof.

12. The method of claim 7, wherein said additional compound is selected from the group consisting of N.sup.6 -benzyl-cAMP and pharmaceutically acceptable salts thereof.

18. A pharmaceutical composition comprising a compound selected from the group consisting of phosphorothioate derivatives of cAMP analogues, wherein said cAMP analogues have been modified at either or both the N.sup.6 or C-8 positions of the adenine moiety, wherein said N.sup.6 - or C-6 substitution is selected from the group consisting of monobenzyl, monoethoxycarbonyl, monobenzoyl, monophenylcarbamoyl, monobutyryl, monophenyl, and diethyl or N-piperidino, and wherein said C-8 substituent is selected from the group consisting of halogen, methylthio, p-chlorophenylthio, .beta.-hydroxyethylamino and methylamino, and pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable carrier.

19. The pharmaceutical composition of claim 18, wherein said compound is a phosphorothioate derivative of 8-halo-cAMP.

20. The pharmaceutical composition of claim 19, wherein said compound is a phosphorothioate derivative of 8-Cl-cAMP, 8-Br-cAMP, or 8-I-cAMP.

21. The pharmaceutical composition of claim 18, wherein said compound is selected from the group consisting of Rp-8-Cl-cAMPS and Sp-8-Br-cAMPS.

22. The pharmaceutical composition of claim 21, wherein said compound is Rp-8-Cl-cAMPS.

23. The pharmaceutical composition of claim 18, which further comprises an additional compound selected from the group consisting of 8-Cl-cAMP, N.sup.6 -benzyl-cAMP, N.sup.6 -benzoyl-8-methylthio-cAMP, 8-methylthio-cAMP, N.sup.6 -benzoyl-cAMP, 8-bromo-cAMP, 8-iodo-cAMP, 8-p-chlorophenyl-thio-cAMP, 8-.beta.-hydroxyethylamino-cAMP, 8-methylamino-cAMP, 8-N,N-dimethylamino-cAMP, N.sup.6 - phenylcarbamoyl-cAMP, N.sup.6 -butyryl-cAMP, N.sup.6,N.sup.6 -phenyl-8-p-chlorophenylthio-cAMP, N.sup.6,N.sup.6 -diethyl-8-p-chlorophenylthio-cAMP, 6-piperidino-8-p-chlorophenylthio-cAMP, N.sup.6 -benzyl-8-benzylthio-cAMP, N.sup.6 -ethoxycarbonyl-cAMP, N.sup.6 -n-butyl-8-p-chlorophenylthio-cAMP, pharmaceutically acceptable salts thereof, and mixtures thereof.

24. The pharmaceutical composition of claim 23, wherein said additional compound is selected from the group consisting of 8-Cl-cAMP and pharmaceutically acceptable salts thereof.

25. The pharmaceutical composition of claim 23, wherein said additional compound is selected from the group consisting of N.sup.6 -benzyl-cAMP and pharmaceutically acceptable salts thereof.

26. A method of inhibiting neoplastic cellular growth in a living mammal afflicted by said neoplastic cellular growth, which comprises administering a compound selected from the group consisting of phosphorothioate derivatives of cAMP analogues, wherein said cAMP analogues have been modified at either or both the N.sup.6 or C-8 positions of the adenine moiety, wherein said N.sup.6 - or C-6 substitution is selected from the group consisting of monobenzyl, monoethoxycarbonyl, monobenzoyl, monophenylcarbamoyl, monobutyryl, monophenyl, and diethyl or N-piperidino, and wherein said C-8 substituent is selected from the group consisting of halogen, methylthio, p-chlorophenylthio, .beta.-hydroxyethylamino and methylamino, and pharmaceutically acceptable salts thereof, in an amount sufficient to provide a serum concentration of about 0.1 to about 100 .mu.M in said mammal and inhibit said neoplastic cellular growth in said mammal.

WEST

Generate Collection | Print

Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. Document ID: US 20020090643 A1

L4: Entry 1 of 8 File: PGPB Jul 11, 2002

PGPUB-DOCUMENT-NUMBER: 20020090643
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020090643 A1

TITLE: COMPOSITIONS AND METHODS FOR MONITORING THE PHOSPHORYLATION OF NATURAL BINDING PARTNERS

PUBLICATION-DATE: July 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
CRAIG, ROGER K.	CHESHIRE		GB	
COYLER, JOHN	WEST YORKSHIRE		GB	

US-CL-CURRENT: 435/7.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020072522 A1

L4: Entry 2 of 8 File: PGPB Jun 13, 2002

PGPUB-DOCUMENT-NUMBER: 20020072522
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020072522 A1

TITLE: Compositions and methods for the treatment of anorectal disorders

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Parks, Thomas P.	San Mateo	CA	US	
Mak, Vivien	Palo Alto	CA	US	
Lee, Jung-Chung	Sunnyvale	CA	US	
Lee, Charles	Union City	CA	US	

US-CL-CURRENT: 514/248; 514/263.31, 514/269, 514/509, 514/565, 514/649

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 3. Document ID: US 20020019002 A1

L4: Entry 3 of 8 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019002
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020019002 A1

TITLE: Methods of monitoring enzyme activity

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Griffiths, Gary	Oldham		GB	

US-CL-CURRENT: 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 4. Document ID: US 20010012856 A1

L4: Entry 4 of 8 File: PGPB Aug 9, 2001

PGPUB-DOCUMENT-NUMBER: 20010012856
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010012856 A1

TITLE: Compositions and methods for the treatment of anorectal disorders

PUBLICATION-DATE: August 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Parks, Thomas P.	San Mateo	CA	US	
Mak, Vivien	Palo Alto	CA	US	
Lee, Jung-Chung	Sunnyvale	CA	US	
Lee, Charles	Union City	CA	US	

US-CL-CURRENT: 514/509; 424/94.4, 514/565

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 5. Document ID: US 6395736 B1

L4: Entry 5 of 8 File: USPT May 28, 2002

US-PAT-NO: 6395736
DOCUMENT-IDENTIFIER: US 6395736 B1

TITLE: Compositions and methods for the treatment of anorectal disorders

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 6. Document ID: US 6391869 B1

L4: Entry 6 of 8 File: USPT May 21, 2002

US-PAT-NO: 6391869
DOCUMENT-IDENTIFIER: US 6391869 B1

TITLE: Compositions and methods for the treatment of anorectal disorders

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 7. Document ID: US 5602110 A

L4: Entry 7 of 8 File: USPT Feb 11, 1997

US-PAT-NO: 5602110
DOCUMENT-IDENTIFIER: US 5602110 A

TITLE: Method and composition for treating cystic fibrosis

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 8. Document ID: US 5439797 A

L4: Entry 8 of 8 File: USPT Aug 8, 1995

US-PAT-NO: 5439797
DOCUMENT-IDENTIFIER: US 5439797 A

TITLE: Detection of analytes using fluorescent energy transfer

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

Generate Collection | Print

Term	Documents
CAMP.DWPI,EPAB,JPAB,USPT,PGPB.	18304
CAMPS.DWPI,EPAB,JPAB,USPT,PGPB.	1217
ANALOG.DWPI,EPAB,JPAB,USPT,PGPB.	345869
ANALOGS.DWPI,EPAB,JPAB,USPT,PGPB.	46550
ANALOGUE.DWPI,EPAB,JPAB,USPT,PGPB.	112259
ANALOGUES.DWPI,EPAB,JPAB,USPT,PGPB.	34929
ANTAGONIST.DWPI,EPAB,JPAB,USPT,PGPB.	40121
ANTAGONISTS.DWPI,EPAB,JPAB,USPT,PGPB.	42359
RP.DWPI,EPAB,JPAB,USPT,PGPB.	20696
RPS.DWPI,EPAB,JPAB,USPT,PGPB.	2069
((2 AND ((CAMP SAME RP) SAME (ANTAGONIST OR ANALOG))) NOT 3).USPT,PGPB,JPAB,EPAB,DWPI.	8
(L2 AND ((CAMP SAME (ANALOG OR ANTAGONIST)) SAME RP) NOT L3).USPT,PGPB,JPAB,EPAB,DWPI.	8

Display Format: - Change Format

Previous Page Next Page

WEST

Generate Collection | Print

Search Results - Record(s) 1 through 10 of 18 returned.

☐ 1. Document ID: US 20020072072 A1

L3: Entry 1 of 18 File: PGPB Jun 13, 2002

PGPUB-DOCUMENT-NUMBER: 20020072072
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020072072 A1

TITLE: ADP-glucose receptor

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Civelli, Olivier	Irvine	CA	US	
Nothacker, Hans-Peter	Irvine	CA	US	
Wang, Zhiwei	Irvine	CA	US	
Reinscheid, Rainer	Irvine	CA	US	

US-CL-CURRENT: 435/7.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020015740 A1

L3: Entry 2 of 18 File: PGPB Feb 7, 2002

PGPUB-DOCUMENT-NUMBER: 20020015740
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020015740 A1

TITLE: Methods and compositions for improving sleep

PUBLICATION-DATE: February 7, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Ackman, C. Bruce	Kingston		CA	
Adams, Michael A.	Kingston		CA	
Heaton, Jeremy P.W.	Gananoque		CA	
Ratz, Jodan D.	Kingston		CA	

US-CL-CURRENT: 424/600

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

☐ 3. Document ID: US 20020006916 A1

L3: Entry 3 of 18 File: PGPB Jan 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020006916
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020006916 A1

TITLE: Promoters of neural regeneration

PUBLICATION-DATE: January 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Song, Hong-jun	La Jolla	CA	US	
Poo, Mu-Ming	La Jolla	CA	US	
Ming, Guo-li	La Jolla	CA	US	
Tessier-Lavigne, Marc	San Francisco	CA	US	
He, Zhigang	San Francisco	CA	US	

US-CL-CURRENT: 514/47; 514/185, 514/231.5, 514/424, 514/509

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

☐ 4. Document ID: US 6440988 B1

L3: Entry 4 of 18 File: USPT Aug 27, 2002

US-PAT-NO: 6440988
DOCUMENT-IDENTIFIER: US 6440988 B1

TITLE: Use of agonists or antagonists of the 5-HT7 receptor to treat disorders of the bladder

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC
Draw Desc | Image

☐ 5. Document ID: US 6432655 B1

L3: Entry 5 of 18 File: USPT Aug 13, 2002

US-PAT-NO: 6432655
DOCUMENT-IDENTIFIER: US 6432655 B1

TITLE: Method of obtaining compositions

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 6. Document ID: US 6395878 B1

L3: Entry 6 of 18 File: USPT May 28, 2002

US-PAT-NO: 6395878
DOCUMENT-IDENTIFIER: US 6395878 B1

TITLE: Nucleic acid encoding a human EP prostaglandin receptor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 7. Document ID: US 6376242 B1

L3: Entry 7 of 18 File: USPT Apr 23, 2002

US-PAT-NO: 6376242
DOCUMENT-IDENTIFIER: US 6376242 B1

TITLE: Methods and compositions for treating platelet-related disorders using MPL pathway inhibitory agents

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 8. Document ID: US 6300087 B1

L3: Entry 8 of 18 File: USPT Oct 9, 2001

US-PAT-NO: 6300087
DOCUMENT-IDENTIFIER: US 6300087 B1

TITLE: DNA encoding a human serotonin receptor (5-HT4B) and uses thereof

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 9. Document ID: US 6268352 B1

L3: Entry 9 of 18 File: USPT Jul 31, 2001

US-PAT-NO: 6268352
DOCUMENT-IDENTIFIER: US 6268352 B1

TITLE: Promoters of neural regeneration

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
Draw Desc | Image

☐ 10. Document ID: US 6268351 B1

L3: Entry 10 of 18 File: USPT Jul 31, 2001

US-PAT-NO: 6268351
DOCUMENT-IDENTIFIER: US 6268351 B1

TITLE: Methods for inducing proliferation in auditory receptor epithelium

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

Generate Collection | Print

Term	Documents
CAMP.DWPI,EPAB,JPAB,USPT,PGPB.	18304
CAMPS.DWPI,EPAB,JPAB,USPT,PGPB.	1217
ANALOG.DWPI,EPAB,JPAB,USPT,PGPB.	345869
ANALOGS.DWPI,EPAB,JPAB,USPT,PGPB.	46550
ANALOGUE.DWPI,EPAB,JPAB,USPT,PGPB.	112259
ANALOGUES.DWPI,EPAB,JPAB,USPT,PGPB.	34929
ANTAGONIST.DWPI,EPAB,JPAB,USPT,PGPB.	40121
ANTAGONISTS.DWPI,EPAB,JPAB,USPT,PGPB.	42359
BR.DWPI,EPAB,JPAB,USPT,PGPB.	701791
BRS.DWPI,EPAB,JPAB,USPT,PGPB.	4728
(2 AND ((CAMP SAME BR) SAME (ANTAGONIST OR ANALOG))).USPT,PGPB,JPAB,EPAB,DWPI.	18
(L2 AND (CAMP SAME (ANALOG OR ANTAGONIST)) SAME BR).USPT,PGPB,JPAB,EPAB,DWPI.	18

Display Format: - Change Format

Previous Page Next Page

WEST

Generate Collection | Print

Search Results - Record(s) 11 through 18 of 18 returned.

☐ 11. Document ID: US 5985585 A

L3: Entry 11 of 18 File: USPT Nov 16, 1999

US-PAT-NO: 5985585
DOCUMENT-IDENTIFIER: US 5985585 A

TITLE: Processes using a human serotonin receptor (5-HT.sub.4B)

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 12. Document ID: US 5968911 A

L3: Entry 12 of 18 File: USPT Oct 19, 1999

US-PAT-NO: 5968911
DOCUMENT-IDENTIFIER: US 5968911 A

TITLE: Method of inducing vasorelaxation to treat pulmonary hypertension

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 13. Document ID: US 5902794 A

L3: Entry 13 of 18 File: USPT May 11, 1999

US-PAT-NO: 5902794
DOCUMENT-IDENTIFIER: US 5902794 A

TITLE: 8-Cl cAMP as anti-cancer drug

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 14. Document ID: US 5843916 A

L3: Entry 14 of 18 File: USPT Dec 1, 1998

US-PAT-NO: 5843916
DOCUMENT-IDENTIFIER: US 5843916 A

TITLE: Cyclic amp analogues, individually and in pairs, to inhibit neoplastic cell

growth

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

---

☐ 15. Document ID: US 5792752 A

L3: Entry 15 of 18 File: USPT Aug 11, 1998

US-PAT-NO: 5792752
DOCUMENT-IDENTIFIER: US 5792752 A

TITLE: 8-chloro camp and related camp compounds as antineoplastic agents

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

---

☐ 16. Document ID: US 5783575 A

L3: Entry 16 of 18 File: USPT Jul 21, 1998

US-PAT-NO: 5783575
DOCUMENT-IDENTIFIER: US 5783575 A

TITLE: Antagonists, their preparation and use

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

---

☐ 17. Document ID: US 5716835 A

L3: Entry 17 of 18 File: USPT Feb 10, 1998

US-PAT-NO: 5716835
DOCUMENT-IDENTIFIER: US 5716835 A

TITLE: Nucleic acid encoding a novel human EP prostaglandin receptor

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

---

☐ 18. Document ID: US 5552267 A

L3: Entry 18 of 18 File: USPT Sep 3, 1996

US-PAT-NO: 5552267
DOCUMENT-IDENTIFIER: US 5552267 A

TITLE: Solution for prolonged organ preservation

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

Generate Collection | Print

Term	Documents
CAMP.DWPI,EPAB,JPAB,USPT,PGPB.	18304
CAMPS.DWPI,EPAB,JPAB,USPT,PGPB.	1217
ANALOG.DWPI,EPAB,JPAB,USPT,PGPB.	345869
ANALOGS.DWPI,EPAB,JPAB,USPT,PGPB.	46550
ANALOGUE.DWPI,EPAB,JPAB,USPT,PGPB.	112259
ANALOGUES.DWPI,EPAB,JPAB,USPT,PGPB.	34929
ANTAGONIST.DWPI,EPAB,JPAB,USPT,PGPB.	40121
ANTAGONISTS.DWPI,EPAB,JPAB,USPT,PGPB.	42359
BR.DWPI,EPAB,JPAB,USPT,PGPB.	701791
BRS.DWPI,EPAB,JPAB,USPT,PGPB.	4728
(2 AND ((CAMP SAME BR) SAME (ANTAGONIST OR ANALOG))).USPT,PGPB,JPAB,EPAB,DWPI.	18
(L2 AND (CAMP SAME (ANALOG OR ANTAGONIST)) SAME BR).USPT,PGPB,JPAB,EPAB,DWPI.	18

Display Format: - Change Format

Previous Page Next Page

WEST

End of Result Set

Generate Collection | Print

L3: Entry 18 of 18 File: USPT Sep 3, 1996

DOCUMENT-IDENTIFIER: US 5552267 A
TITLE: Solution for prolonged organ preservation

Drawing Description Text (3):
FIGS. 2A-B. Augmentation of the cAMP second messenger pathway enhances cardiac preservation. (A) Use of cAMP analogs and related controls. Rat hearts were explanted and preserved (as above) for 12 hours using solutions containing LR supplemented with either cAMP analogs, db-cAMP (4 mM) or 8-Br-cAMP (8 brcAMP, 4 mM), sodium butyrate (but, 4 mM), or 8-bromoadenosine (8ba, 4 mM). The percentage of surviving grafts after reimplantation is shown. (B) 8-Br-cAMP dose response experiment at 12 hours preservation demonstrates maximal benefit is achieved at a concentration of 0.1 mM. (C) Effect of phosphodiesterase inhibitors on cardiac preservation. Hearts were explanted and preserved for 12 hours as described above. Preservation solutions include LR alone, and LR supplemented with indolidan (ind; 10 .mu.M) or rolipram (rol; 10 .mu.M). (D) The effects of cAMP-dependent protein kinase agonists and inhibitors on cardiac preservation. Hearts were explanted and preserved for 12 hours as above in either LR, LR+8-Br-cAMP 11 mM), or LR+8-Br-cAMP (0.1 mM) +the cAMP-dependent protein kinase inhibitor R.sub.p -cAMPS (0.5 mM). In other experiments, the cyclic AMP-dependent protein kinase agonist S.sub.p -cAMPS (0.5 mM) was added to LR. Bars represent the percentage of surviving grafts, and demonstrate the importance of the cAMP-dependent protein kinase with respect to preservation. [** denotes p<0.01 compared with LR alone; preservation scores parallelled survival data in all instances and are not shown].

Detailed Description Text (14):
Phosphodiesterase (PDE) inhibitors are commonly categorized according to five families (See, W. J. Thompson, Pharmac. Ther. (1991) 51: 13-33; and J. P. Hall, Br. J. clin. Pharmac. (1993) 35: 1-7) (However, this classification is not universal and other classification schemes can be found in the literature.): PDE I--Ca.sup.+2 /Calmodulin-activatable; PDE II--cGMP activatable; PDE III--cGMP inhibitable; PDE IV--cAMP-specific; PDE V--cGMP-specific. These families include, but are not limited to, the following phosphodiesterase inhibitors:

Detailed Description Text (84):
The following reagents were obtained from the indicated commercial sources; D-glucose, magnesium sulfate, dextran (M.W. 308,000 daltons), monopotassium phosphate (KH.sub.2 PO.sub.4), potassium gluconate, adenosine, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), N-acetylcysteine (N--AC), and heparin (porcine intestinal, 100000 u/g) were obtained from Sigma Chemical Company (St. Louis, Mo.). Dibutyryl adenosine 3',5'-cyclic monophosphate (dibutyryl cyclic AMP, or db cAMP) was obtained from Aldrich Chemical Company, Incorporated (Milwaukee, Wis.). Verapamil (2.5 mg/ml) was obtained from Knoll Pharmaceutical Co. Nitroglycerin (5 mg/ml) was obtained from DuPont (Manati, Puerto Rico). Cefazolin (400 mg/ml) was obtained from Lyphomed (Rosemont, Ill.). Lactated Ringer's solution was obtained from Abbott (North Chicago, Ill.).

Detailed Description Text (133):
In view of the ischemia/hypoxia which accompanies harvest and storage of an explanted heart, it was hypothesized that addition of cAMP analogs to preservation solutions would exert a beneficial effect on vascular function and organ preservation. The results of these studies indicate that agents which raise

intracellular cAMP (such as rolipram or indolidan) or cAMP analogs/agonists (including N.sup.6,O.sup.2' -dibutyryl cAMP [db-cAMP], 8bromoadenosine3', 5'monophosphate [8-Br-cAMP], and S.sub.p -cAMPS) promote cardiac preservation in a rat heterotopic transplant model. cAMP analogs appear to exert their effects via a pathway that involves, at least in part, stimulation of cAMP-dependent protein kinase, which results in enhanced blood flow and decreased leukocyte infiltration of the graft. To further explore the role of the vasculature in cAMP-induced enhancement of graft preservation, vascular smooth muscle cells (SMCs) were subjected to hypoxia, to determine whether a similar decline in intracellular cAMP would occur in vascular SMCs exposed to hypoxia as has been reported for ECs (25). In this series of experiments, it is demonstrated that vascular SMCs exposed to hypoxia show a time-dependent decline in cAMP levels, associated with an increase in phosphodiesterase activity, notably types III and IV. These findings are important because SMCs, as the cellular end-regulators of blood flow, are critical to vascular homeostasis, and because cAMP is known to play an important role in the regulation of vasomotor tone (27). Taken together, these studies indicate that a new level of intervention, addition of cAMP analogs to preservation solutions for maintenance of vascular function, improves cardiac preservation after prolonged hypothermic storage.

Detailed Description Text (148):
In order to establish that the cAMP was responsible for improving graft survival, and knowing that when db-cAMP enters the cell it is converted to N.sup.6 -monobutyryladenosine 3'5'monophosphate(mb-cAMP) and butyrate (40), the effect of sodium butyrate alone was assessed (FIG. 2A). Hearts preserved with butyrate in place of db-cAMP consistently failed after a 12 hr preservation period. Another cAMP analog, 8-Br-cAMP prolonged graft survival, whereas 8-bromoadenosine was ineffective. When 8-Br-cAMP was tested over a broad range of concentrations during a 12 hr preservation period, it was effective at concentrations .apprxeq.10-times lower than db-cAMP (FIG. 2B): 100% of grafts survived when preserved in LR solution containing 8-Br-cAMP at 0.1 mM. Further evidence in support of cyclic AMP's role in successfully prolonging the preservation period come from the results of experiments in which the phosphodiesterase inhibitors rolipram (10 .mu.M) or indolidan (10 .mu.M) (41) uniformly enhanced preservation of hearts stored for 12 hrs in LR solution (FIG. 2C). These data suggest that elevation of endogenous cAMP levels has a similar beneficial effect to exogenously administered cyclic AMP analogs.

Detailed Description Text (149):
An important means through which cAMP exerts its effects intracellularly is via stimulation of the cAMP-dependent protein kinase (PKA). To explore the potential relevance of this mechanism to enhanced cardiac preservation, two stereoisomers of adenosine 3'5'monophosphorothioate were used (42). The S.sub.p -isomer (S.sub.p -cAMPS) is a PKA agonist that is not hydrolyzable by cellular enzymes, whereas the R.sub.p -isomer (R.sub.p -cAMPS) is a nonhydrolyzable competitive PKA antagonist, which binds to the PKA without causing activation. S.sub.p -cAMPS enhanced preservation, whereas R.sub.p -cAMPS abolished the salutory effect of 8-Br-cAMP on preservation, consistent with the important role of the PKA pathway in preservation enhancement (FIG. 2D). Although the cyclic GMP-dependent protein kinase may have a role in cardiac preservation, its direct activation by cyclic AMP analogs is unlikely since mb-cAMP and 8-Br-cAMP are respectively 313-fold and 53-fold less potent than cGMP in activating the cGMP-dependent protein kinase (43), and Sp-cAMPS is an antagonist of the cGMP-dependent protein kinase (44).

Detailed Description Text (230):
67. Simpson, P., J. Schelm, J. Smallwood, M. Clay, and T. Lindstrom. 1992. Inhibition of granulocyte cAMP-phosphodiesterase in vivo is not sufficient to protect the canine myocardium from reperfusion injury. J. Cardiovasc. Pharmacol. 19: 987-955.

Detailed Description Text (319):
Like cGMP, cAMP is a vasodilator.sup.61 which can interfere with neutrophil adhesion to endothelium.sup.62. Because of the intricate interrelationships between cAMP and cGMP metabolism (such as cGMP-stimulatable and cGMP-inhibitable phosphodiesterase activities).sup.63, it is impossible to completely exclude a role for elevation of intracellular cAMP in the enhancement of pulmonary preservation observed in these

studies. The importance of endogenous cGMP in this setting, however, comes from several lines of evidence. In these studies, cGMP levels were reduced in reperfused lungs. The analog of cGMP that was used in this study (8-Br-cGMP) is 500 times more potent at stimulating the cGMP dependent protein kinase compared with the cAMP-dependent protein kinase.sup.64,65. In addition, 8-Br-cGMP does not interact with the allosteric binding sites on the cGMP-regulated phosphodiesterases.sup.66, and hence does not effect cAMP hydrolysis in intact cell studies. Because the cGMP-specific (type V) phosphodiesterase inhibitor M&B 22948 augments the pulmonary vasodilating effects of NO(cGMP)-dependent vasodilators, but not those of a cAMP-dependent vasodilator such as isoproterenol.sup.38, this suggests that its beneficial vascular effects in this study are likely to have been cGMP-mediated. Finally, the Rp-8-pCPT-cGMPS compound that blocked preservation enhancement in this study is relatively specific for the cGMP-dependent protein kinase (K.sub.i 0.5 .mu.M, vs K.sub.i 8.3 .mu.M for the cAMP-dependent protein kinase, Dr. H-G Ginesier, personal communication).sup.39, suggesting that its ability to block the effects of 8-Br-cGMP on lung preservation was not due to blocking cAMP-dependent protein kinase. Our results with 8-Br-cGMP are consistent with previous studies demonstrating that acidified sodium nitrite, nitroglycerin, and other agents which elevate intracellular cGMP blunt myocardial injury and neutrophil accumulation during reperfusion.sup.67-69. The potential applicability of these findings to clinical transplantation is suggested by the ability of 8-Br-cGMP to enhance lung preservation even in the currently accepted clinical standard lung preservation solution, modified Eurocollins. These studies suggest that augmentation of the NO pathway at the level of cGMP will provide a novel pharmacological approach to normalize vascular function in the critical early stages following reperfusion.

Detailed Description Text (486):
65. Heuze-Joubert I, Mennecier P, Simonet S, Laubie M, Verbeuren T J: Effect of vasodilators, including nitric oxide, on the release of cGMP and cAMP in the isolated perfused rat kidney. Eur J Pharmacol 1992;220:161-171

Detailed Description Text (487):
66. Nolte C, Eigenthaler M, Schanzenbacher P, Walter U: Comparison of vasodilatory prostaglandins with respect to cAMP-mediated phosphorylation of a target substrate in intact human platelets. Biochem Pharmacol 1991;42:253-262

L3: Entry 10 of 18 File: USPT Jul 31, 2001

DOCUMENT-IDENTIFIER: US 6268351 B1
TITLE: Methods for inducing proliferation in auditory receptor epithelium

Brief Summary Text (7):
The present invention provides a novel system for the regeneration of auditory receptor hair cells. The generation of replacement hair cells following damage by sound or drugs has been clearly demonstrated in birds where the hair cells arise from a normally non-dividing supporting cell population induced to proliferate by the damage. The present invention demonstrates that agents which increase cAMP levels induce cell proliferation in explanted but otherwise undamaged receptor epithelium of cochleas, and that protein kinase A (PKA) inhibitors block this proliferative response. Furthermore, the proliferative response which follows in vitro gentamicin damage is also significantly blocked by PKA inhibitors. Accordingly, biochemical manipulation of the cAMP pathway is now possible. Thus, in one embodiment of the invention, a research tool is provided for the elucidation of the molecular events involved in the restoration of auditory receptor hair cells. In a preferred embodiment, stimulation of proliferation is achieved via the administration of agents which activate the cAMP pathway, such as stimulators of adenylate cyclase (i.e., forskolin). Forskolin may be used in a range of about 1-200 .mu.M. Narrower ranges of forskolin concentration are also contemplated in practicing the present invention, i.e, between about 10 .mu.M-100 .mu.M, or between about 75 .mu.M-150 .mu.M. Alternatively, cAMP levels may be augmented by direct delivery of cAMP or analogues thereof (i.e., 8-Br-cAMP). Proliferation of auditory receptor cells may be assessed by co-administration and detection of agents that are incorporated into replicating DNA (i.e., Bromo-deoxyuridine).

Brief Summary Text (9):
In yet another embodiment of the invention, a method for treating a patient suffering from hearing loss is provided. The term "patient" as used herein may be a human or an animal subject. The method comprises delivering to a patient's auditory receptor epithelium, a cAMP activating agent in a suitable pharmaceutical carrier in an effective amount to activate the signal transduction pathway responsible for regeneration of hair cells in the cochlea epithelium. In an alternative embodiment of the invention, the method is used to assess the regeneration of hair receptor cells in the bird. In this embodiment, an agent for detecting DNA replication is co-administered to the damaged auditory epithelium with the cAMP pathway activating agent. Following stimulation of a proliferative response, the animal is sacrificed and the level of proliferation assessed.

Detailed Description Text (19):
The first set of experiments was performed using undamaged cochleas. Five groups of cochleas were explanted from four day-old chicks and immediately placed in culture in the presence of BrdU. The first group was treated with no additional pharmacological agent, and served as a control. The second group was treated with the adenyl cyclase activator forskolin in order to increase cAMP levels. The third group was treated with the membrane-permeable cAMP analog 8-bromo-cAMP and the phosphodiesterase inhibitor iso-butyl methylxanthine (IBMX) as an independent method of activating the cAMP pathway. The fourth group was treated with both forskolin and the specific PKA inhibitor H89. The fifth group was treated with both forskolin and the specific PKA inhibitor KT5720. Groups four and five were included to confirm that any proliferative response was mediated by PKA, the major cAMP pathway effector. After incubation for three days, cochleas were fixed and whole mounts

examined for BrdU incorporation using immunohistochemistry.

Detailed Description Text (30):
The results presented implicate the involvement of the cAMP pathway in the regeneration of auditory receptor epithelium. Accordingly, hearing compromised patients would be treated with agents that stimulate cAMP production. Fo example, forskolin (25-100 .mu.M dissolved in dimethylsulfoxide (DMSO)) in a suitable pharmaceutical carrier would be delivered via drops, injection or to the cochlea of the patient. Alternatively, the drug delivery system may comprise an osmotic pump or other timed-release gel delivery systems known in the art. Restoration of hearing would then be assessed using standard methods.

Detailed Description Text (59):
25. Hentrich, F., Gothert, M. & Greschuchna, D. Involvement of cAMP in modulation of noradrenaline release in the human pulmonary artery. Naunyn Schmiedebergs. Arch. Pharmacol. 330, 245-247 (1985).

WEST

Generate Collection | Print

L3: Entry 14 of 18 File: USPT Dec 1, 1998

US-PAT-NO: 5843916
DOCUMENT-IDENTIFIER: US 5843916 A

TITLE: Cyclic amp analogues, individually and in pairs, to inhibit neoplastic cell growth

DATE-ISSUED: December 1, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cho-Chung; Yoon Sang	Bethesda	MD		
Jastorff; Bernd	Oyten			DE
Genieser; Hans-Gottfried	Bremen			DE

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The United States of America as represented by the Department of Health and Human Services	Washington	DC			06

APPL-NO: 08/ 225097 [PALM]
DATE FILED: April 8, 1994

PARENT-CASE:
This is a continuation of application Ser. No. 07/877,523 filed on May 1, 1992, now abandoned.

INT-CL: [06] A61 K 31/70, C07 H 19/213

US-CL-ISSUED: 514/47; 536/26.13
US-CL-CURRENT: 514/47; 536/26.13

FIELD-OF-SEARCH: 514/47, 536/26.13

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	3849397	November 1974	Robins, I et al.	536/26.13
☐	3948886	April 1976	Shuman et al.	536/26.12
☐	4058659	November 1977	Robins, II et al.	536/26.13
☐	4208406	June 1980	Lapinet et al.	514/47

OTHER PUBLICATIONS

Genieser et al.(II), "Derivatives of 1.beta.-D-ribofuranosylbenzimidazole 3', 5'-phosphate That Mimic the Actions of Adenosine 3 ', 5'-phosphate (cAMP) and Guanosine 3', 5'-phosphate (cGMP),"Carbohydrate Research,234, 217-235 (1992).
Kano et al., "The Activation of cAMP-dependent Protein Kinase Is Directly Linked to the Reulation of Osteoblast Proliferation (UMR-106) by Parathyroid Hormone,"Biochem. Biophys. Res. Comm., 177(1), 365-369 (1991).
Yokozaki et al., "Unhydrolyzable Analogues of Adenosine 3:5 -Monophosphate Demonstrating Growth Inhibition and Differentiation in Human Cancer Cells, " Cancer Research,52, 2504-2508 (1992).
Beardsley, "Trends in Cancer Epidemiology; A War Not Won," Scientific American 270 (1), 130-138 (1994)
Ally et al., "Selective Modulation of Protein Kinase Isozymes by the Site-Selective Analog 8-Chloroadenosine 3', 5'-Cyclic Monophosphate Provides a Biological Means for control of Human Colon Cancer Cell Growth," Proc. Nat. Acad. Sci. USA,85, 6319-6322 (Sept. 1988).
Clair et al., "Site-Selective cAMP Analogs Induce Nuclear Translocation of the RII cAMP Receptor Protein in Ha-MuSV-Transformed NIH/ 3T3 Calls," FEBS Letters, 224(2), 337-384 (Nov.1987).
Cho-chung et al.(I), "Site-Selective Cyclic AMP Analogs as New Biological Tools in Growth Control, Differentiation, and Proto-oncogene Regulation," Cancer Invest.,7(2), 161-177 (1989).
Cho-chung et al.(II),"Role of Site-Selective cAMP Analogs in the Control and Reversal of Malignancy,"Pharmac. Ther., 50, 1-33 (1991).
Cho-chung et al.(III),"Role Cyclic AMP Receptor Proteins in Growth, Differentiation, and Suppression of Malignancey: New Approaches to Therapy, " Cancer Res.,50, 7093-7100 (Nov. 1990).
Cho-Chung(II), "Site-Selective 8-Chloro-Cyclic Adenosine 3 ', 5'-Monophosphate as a Biologic Modulator of Cancer: Restoration of Normal Control Mechanisms," J. Nat'l Cancer Inst.,81 (13), PP. (July 5, 1989).
Cho-chung et al.(IV), "In Vivo Inhibition of Growth of Two Hormone Dependent Mammary Tumors by Dibutyryl1 Cyclic AMP," Science,183, 87-88 (Jan. 1974).
Dostmann et al., "Probing the Cyclic Nucleotide Binding Sites of cAMP-Dependent Protein Kinases I and II with Analogs of Adenosine 3',5'-Cyclic Phosphorothioates, " J. Biol. Chem.,265(18), 10484-10491 (1990).
Geniesser et al., "Synthesis of Nucleoside-3', 5'-Cyclic Phosphorothioates by Cyclophosphorylation of Unprotected Nucleosides, " Tetrahedron Letters,29(23), 2803-2804 (1988).
Helson et al., "A Rationale for the Treatment of Metastatic Neuroblastoma," J. Nat'l Cancer Inst.,57(3), 727-729 (Sept. 1976).
Holmgren et al., "In Vivo Modulation of Intracellular cAMP and Cell Growth of a Lymphatic Tumour in Mice by Cholera Toxin," Exp. Cell Res.,108, 31-39 (1977).
Katsaros et al., "Site-Selective Cyclic AMP Analogs Provide a New Approach in the Conrol of Cancer Cell Growth," FEBS Letters,223(1), 97-103 (Oct. 1987).
O'Brian et al., "A Kinetic Study of Interactions of (Rp)-and (Sp)-Adenosine Cyclic 3', 5'-Phosphorothioates with Type II Bovine Cardiac Muscle Adenosine Cyclic 3', 5'-Phosphate Dependent Protein Kinase," Biochemistry,21, 4371-4376 (1982).
Ogreid et al.(I), "Activation of Protein Kinase Isozymes by Cyclic Nucleotide Analogs Used Singly or in Combination," Eur. J. Biochem., 150, 219-227 (1985).
Ogreid et al.(I), "Comparison of the Two Classes of Binding Sites (A and B) of Type I and Type II Cyclic-AMP-Dependent Protein Kinases by Using Cyclic Nucleotide Analogs, ", Eur. J. Biochem., 181, 19-31 (1989).
Ogreid et al.(I),"Comparison of the Two Classes of Binding Sites (A and B) of Type I and Type II Cyclic-AMP-Dependent Protein Kinases by Using Cyclic Nucleotide Analogs," Eur. J. Biochem., 181, 19-31 (1989).
Cho-Chung et al.(V), "Site-Selective cAMP Analogs are Cytostatic and Differentiating Agents for a Spectrum of Human Cancer Cell Lines: Potential for Application to Chemotherapy," Proc. of ASCO, 6, Abstract No. 62 (Mar. 1987).
Prasad, "Differentiation of Neuroblastoma Cells in Culture," Biol. Rev.,50, 129-265 (1975).
Rannels et al., "Two Different Intrachain cAMP Binding Sites of cAMP-Dependent Protein Kinases," J. Biol. Chem., J. Biol, Chem.,255(15), 7085-7088 (1980).
Robinson-Steiner et al., "Probable Involvement of Both Intrachain cAMP Binding Sites

in Activation of Protein Kinase," J. Biol. Chem., 258(2), 1032-1040 (1983).
Tagliaferri et al.(I), "Two Classes of cAMP Analogs Synergistically Inhibit p21 ras Protein Synthesis and Phenotypic Transformation of NIH/3T3 Cells Transfected with Ha-MuSV DNA," Biochem. Biophys. Res. Comm.,130(3), 1193-1200 (Aug. 1985).
Tagliaferri et a.(II), "Synergistic Inhibition of Growth of Breast and Colon Human Cancer Cell lines by Site-Selective Cyclic AMP Analogs, "Cancer Res., 48, 1642-1650 (Mar. 1988).
Tagliaferri et al.(III), "Reverse Transformation of Harvey Murine Sarcoma Virus-Transformed NIH/3T3 Cells by Site-Selective Cyclic AMP Analogs,"J. Biol, Chem., 263 (1), 409-416 (1988)
Tortora et al., "Site-Selective cAMP Analogs at Micromolar Concentrations Induce Growth Arrest and Differentiation of Acute-Promyelocytic, Chronic Myelocytic, and Acute Lymphocytic Human Leukemia Cell Lines," Blood,71(1), 230-233 (Jan. 1988).
Tsuji et al., "Neuronal Differentiation of Oat Cell Carcinoma In Vitro by Dibutyryl Cyclic Adenosine 3',5'-Monophosphate,"Cancer Letters, 1, 311-318 (1976).

ART-UNIT: 121

PRIMARY-EXAMINER: Kight, III; John

ASSISTANT-EXAMINER: Crane; L. Eric

ABSTRACT:

A method of inhibiting the proliferation of cells, particularly cancerous cells, by contacting the cells with a phosphorothioate derivative of a cAMP modified at either or both the C-6 and C-8 positions of the adenine moiety, and pharmaceutical compositions therefor.

37 Claims, 20 Drawing figures

L3: Entry 12 of 18 File: USPT Oct 19, 1999

DOCUMENT-IDENTIFIER: US 5968911 A
TITLE: Method of inducing vasorelaxation to treat pulmonary hypertension

Brief Summary Text (4):
Initial observations dealing with the use of cAMP and cGMP compounds go back to models of heart transplantation, where it was demonstrated that these systems were dysfunctional in the blood vessels of a transplanted heart. Supplementation of either the cGMP or the cAMP pathways could enhance the function of blood vessels within the graft, promoting successful transplantation. Stimulators of cAMP pathway used in these experiments included Sp-cAMPs, 8-Br-cAMP, db-cAMP, and phosphodiesterase inhibitors (indolidan, rolipram), all of which helped graft preservation. An antagonist of this pathway (RpcAMPS) blocked the beneficial effects of 8-Br-cAMP.

Detailed Description Text (22):
Phosphodiesterase (PDE) inhibitors are commonly categorized according to five families (See, W. J. Thompson, Pharmac. Ther. (1991) 51: 13-33; and J. P. Hall, Br. J. clin. Pharmac. (1993) 35: 1-7) (However, this classification is not universal and other classification schemes can be found in the literature.): PDE I - Ca.sup.+2 /Calmodulin-activatable; PDE II - cGMP activatable; PDE III - cGMP inhibitable; PDE IV - cAMP-specific; PDE V - cGMP-specific. These families include, but are not limited to, the following phosphodiesterase inhibitors:

Detailed Description Text (33):
There are currently no effective therapies for pulmonary hypertension. Although the gas nitric oxide (NO) selectively dilates the pulmonary vascular bed, it is difficult to administer, has a short biologic half-life, and is potentially toxic. It was hypothesized that stimulation of the nitric oxide pathway using a nonhydrolyzable, membrane permeant analog of cGMP such as 8-Br-cGMP (7,8,9) or Sp-8-Br-cGMPS, or the cAMP analogs dibutyryl cAMP or 8-Br-cAMP which are administered via inhalation would confer relative pulmonary selectivity and circumvent the difficulties associated with administration of NO. These studies using three porcine models of pulmonary hypertension demonstrate the potential therapeutic usefulness of administering cGMP/cAMP analogs for the treatment of pulmonary vasoconstrictive disorders. Pulmonary hypertension was induced in 23 pigs by an intravenous thromboxane A.sub.2 analog, U-46619, (Tx, n=9), hypoxic ventilation (H, n=8), or oleic acid (OA, n=6). Because NO increases cyclic guanosine 3'-5'-monophosphate (cGMP) levels in vascular smooth muscle, tests were done to determine whether inhalation of a membrane permeable cGMP analog to cause the highest possible pulmonary concentrations could cause selective pulmonary vasodilation in several models of pulmonary hypertension. In a comparison of aerosolized intratracheal inhalation of 8Br-cGMP with physiologic saline, pulmonary vascular resistance (PVR) declined by 24.+-.3.8% ($p<0.001$), 28.+-.3.7% ($p<0.01$), and 34.+-.8.1% ($p<0.05$), for the Tx, H, and OA models, respectively. This compares favorably with PVR reduction seen following inhaled NO (50 ppm) (.DELTA.PVR for 8-Br-cGMP was 50% of the .DELTA.PVR for NO in the Tx model). The declines in systemic vascular resistance (SVR) following intratracheal 8Br-cGMP (1.+-.4% and 9.+-.4%, respectively) was significantly less ($P<0.01$) than the declines in PVR in the Tx and H models, but were similar in the OA model. Intravenous 8Br cGMP lowers PVR and SVR to a similar degree. 8Br-cGMP lowers PVR in a time and dose-dependent manner, with maximal effect achieved after one hour at doses as low as 0.03 .mu.g/kg. The selective decline in PVR was not mimicked by inhalation of

guanosine-5'monophosphate, suggesting that stimulation of the NO/cGMP pathway beyond the level of NO results in selective pulmonary vasodilation independent of stimulation of purine receptors. Pressure-volume loops constructed at different preloads using an intraventricular conductance catheter demonstrate little effect of inhaled 8Br-cGMP on ventricular contractility, suggesting that this agent may be given safely in the setting of cor pulmonale. These studies demonstrate that inhalation of an agent which is an analog of cGMP can selectively reduce PVR, and may be useful in pulmonary vasoconstrictive diseases.

Detailed Description Text (36):
Hemodynamics were recorded at end expiration at baseline and every 10-15 minutes thereafter, and included measurements of heart rate (HR, beats/min), central venous pressure (CVP, mm Hg), pulmonary artery wedge pressure (PCWP, mm Hg), mean arterial and mean pulmonary arterial pressures (MAP, MPAP, mm Hg), and thermodilution cardiac outputs (CO, L/min). Three repetitive measurements of cardiac output using iced saline injection were averaged for each time point. When a stable baseline PVR was demonstrated, pulmonary hypertension was induced by 1) continuous intravenous infusion of the thromboxane A.sub.2 analog (9,11-dideoxy-11.alpha.,9.alpha.-epoxymethanoprostaglandin F.sub.2.alpha. (10) (Sigma Chemical Co., St. Louis, Mo.) at a rate which resulted in a mean PA pressure of .apprxeq.30 mm Hg (0.07-0.11 .mu.g/kg/min); 2) ventilation with a hypoxic gas mixture containing oxygen and nitrogen with the proportion of oxygen titrated to a mean PA pressure .apprxeq.30 mm Hg, with continuous hypoxia monitored by inhaled (.apprxeq.10%) and arterial (paO.sub.2) .apprxeq.35 mm Hg) oxygenation; 3) intravenous infusion of oleic acid (Sigma) 0.3 ml/kg over 1 hour. After stable measurements of PVR in the hypertensive state were achieved, aerosolized physiologic saline (0.9% sodium chloride) was given endotracheally, followed by at least one hour of observation. After observing consistent measurements of PVR, 8Br-cGMP (Sigma) was then given endotracheally (0.03-614 .mu.g/kg in a 5 ml volume of physiologic saline, administered over 5 minutes) and hemodynamic data were recorded every 10-15 minutes. The normal saline and test compounds dissolved in normal saline were delivered endotracheally as a mist. Intravenous administration consisted of dissolving 300 .mu.g/kg 8Br-cGMP in 5 ml of physiologic saline and injecting the solution as a bolus. In other experiments, 8-bromoguanosine-5'monophosphate (272 .mu.g/kg, Sigma), Sp-cGMPS (Biolog, La Jolla, Calif.), dibutyryl cAMP (db-cAMP) or 8-Br-cAMP were similarly administered. NO (Airco, Lodi, N.J.) at a concentration of 50 ppm was administered during controlled ventilation after repeated measurements indicated unchanging pulmonary hypertension. At least 10 minutes of continuous NO inhalation elapsed before hemodynamic measurements were taken.

The Journal of Immunology

The Journal of Immunology, 1999, 162: 1178-1185.

▷ Abstract of this Article
▷ **Reprint (PDF) Version of this Article**
▷ Similar articles found in:
The JI
PubMed
▷ PubMed Citation
▷ This Article has been cited by:
other online articles
▷ Search Medline for articles by:
Aukrust, P. || Müller, F.
▷ Alert me when:
new articles cite this article
▷ Download to Citation Manager

Increased Activation of Protein Kinase A Type I Contributes to the T Cell Deficiency in Common Variable Immunodeficiency[1]

Pål Aukrust[2,*], Einar Martin Aandahl[†], Bjørn S. Skålhegg[†], Ingvild Nordøy[*], Vidar Hansson[†], Kjetil Taskén[†], Stig S. Frøland[*] and Fredrik Müller[*]

[*] Section of Clinical Immunology and Infectious Diseases and Research Institute for Internal Medicine, Medical Department A, Rikshospitalet, Oslo, Norway; and [†] Institute of Medical Biochemistry, University of Oslo, Oslo, Norway

▷ Abstract

△ Top
▪ Abstract
▽ Introduction
▽ Materials and Methods
▽ Results
▽ Discussion
▽ References

The molecular mechanisms underlying the T cell dysfunction often present in common variable immunodeficiency (CVI) are not established. cAMP-dependent protein kinase A type I (PKAI) is an important inhibitor of T cell proliferation after Ag stimulation. We therefore investigated the possibility that activation of PKAI may be involved in the development of T cell dysfunction in CVI. An exogenously added PKAI-selective antagonist (Rp-8-Br-cAMPS) induced a significant increase in anti-CD3-stimulated PBMC proliferation in 20 CVI patients compared with no effect in 15 controls. Purified T cells from 7 CVI patients with strictly defined T cell deficiency had elevated endogenous cAMP levels compared with controls. Treatment of T cells from these CVI patients with Rp-8-bromo-cAMP-phosphorothioate markedly improved anti-CD3-stimulated proliferation (up to 3.7-fold), particularly in $CD4^+$ lymphocytes, reaching proliferation levels comparable to control values. No effect of cAMP antagonist on T cell proliferation was seen in controls. In these CVI patients, cAMP antagonist also increased IL-2 production in anti-CD3-stimulated T cells. However, exogenously added IL-2 at concentrations comparable to the achieved increase in IL-2 levels after addition of cAMP antagonist had no effect on T cell proliferation. Furthermore, the stimulatory effects of exogenously added IL-2 at higher concentrations and cAMP antagonist on T cell proliferation were additive. Our findings indicate that increased PKAI activation may be an important molecular basis for the T cell defect in CVI and suggest that the cAMP/PKAI system may be a potential molecular target for

immunomodulating therapy in these patients.

Introduction

Cyclic AMP is a key negative regulator of lymphocyte proliferation (1, 2, 3). With few exceptions all known actions of cAMP are mediated through cAMP-dependent protein kinases (PKA)[3] (4, 5). Enhanced cAMP levels completely abolish early tyrosine phosphorylation following engagement of the Ag receptor as well as T cell proliferation induced through the TCR/CD3 complex (2, 3). This regulation seems mainly to be mediated through PKA type I (PKAI), and this isoenzyme of PKA redistributes and colocalizes with the Ag receptor during T cell activation (6). This serves to establish PKAI as an acute negative modulator of T cell Ag responses and clonal expansion. Triggering of the TCR/CD3 complex in itself leads to the production of cAMP (7, 8), suggesting that PKA activation following TCR/CD3 stimulation may represent a negative feedback control mechanism. In support of this, impaired PKAI has recently been demonstrated in T cells from patients with systemic lupus erythematosus, possibly contributing to persistent immune activation in these patients by lack of inhibition (9).

Common variable immunodeficiency (CVI) is a heterogeneous group of B cell deficiency syndromes characterized by defective Ab production, recurrent sinopulmonary bacterial infections, and a high rate or incidence of lymphoid and gastrointestinal malignancies, nonmalignant lymphoid hyperplasia, and granulomatous inflammation (10, 11). Although B cell defects are the immunologic hallmark of CVI, T cell abnormalities such as abnormal distribution of T cell subsets, impaired proliferative response to recall Ags, and dysregulated cytokine production, have been reported in a significant subset of patients (12, 13). These T cell abnormalities may be of importance both for the defective Ab production and for the clinical manifestations in CVI. In fact, the finding that B cells from CVI patients can proliferate and produce Igs if appropriately stimulated in vitro (14, 15) suggests that B cells in many CVI patients may not be intrinsically defective and that inappropriate T cell help is of importance for induction of the immunodeficiency.

The molecular mechanisms underlying the T cell dysfunction in CVI are not established. However, studies in a subgroup of CVI patients have suggested a defect in the early phase of T cell activation after triggering the TCR/CD3 complex and before activation of protein kinase C (PKC) (16, 17, 18). As cAMP through activation of PKAI exerts an early inhibitory effect on signaling through the TCR/CD3 complex (3, 19, 20), we investigated the possibility that the cAMP/PKAI system may be involved in the development of T cell dysfunction in CVI.

Materials and Methods

Top
Abstract
Introduction
Materials and Methods
Results
Discussion
References

Patients and controls

Twenty consecutively recruited patients with the diagnosis of CVI based on established criteria (10, 11) were included in the study (Table ⊡). Based on previously defined criteria (21, 22), 10 patients had splenomegaly, 6 had chronic rhinosinusitis, 3 had nodular intestinal lymphoid hyperplasia, 3 had bronchiectasis, and 3 had autoimmune disorders. All patients were treated with s.c. self-administered Ig, and all had IgG levels >5.0 g/l during the last 3 mo before blood collection. Blood samples for the study were drawn just before Ig administration. At the time of blood collection, all patients were without any manifestations of acute infection or acute exacerbation of chronic disease. None was receiving antibiotics or immunosuppressive drugs. Controls in the study were 15 sex- and age-matched healthy blood donors (Table ⊡).

View this table: [in this window] [in a new window]

Table I. Clinical and immunologic characteristics of the study group[1]

Isolation of cells

PBMC were obtained from heparinized blood by Isopaque-Ficoll (Lymphoprep, Nycomed Pharma, Oslo, Norway) gradient centrifugation within 45 min after blood sampling (23). Further negative selection of $CD3^+$ T cells by monodisperse immunomagnetic beads was performed at 4°C. PBMC suspended in PBS with 0.3% BSA (Calbiochem, La Jolla, CA), were mixed with beads coated with Abs to CD14 (Dynabeads M-450 CD14, Dynal, Oslo, Norway), CD19 (Dynabeads M-459 Pan B, Dynal), and CD56 (clone B159, PharMingen, San Diego, CA; bound to beads precoated with rat anti-mouse IgG1, Dynal) in a cell-to-bead ratio of 1:10 and were placed on a rocking platform for 45 min. After removal of rosetted cells by application of a magnet (Dynal), negatively selected cells were washed twice in cold PBS/0.3% BSA and either immediately stored in liquid nitrogen (for cAMP analyses) or used for further functional studies. Cells were routinely screened by flow cytometry and were shown to consist of >90% $CD3^+$ T cells and low levels of monocytes ($CD14^+$ cells, <2%), B cells ($CD19^+$ cells, <2%), and NK cells ($CD56^+$ cells, <5%).

Lymphocyte proliferation assay

PBMC (10^6/ml, 200 μl/well) and $CD3^+$ T cells (0.75×10^6/ml, 100 μl/well), suspended in RPMI 1640 (Life Technologies, Paisley, U.K.) with 2 mmol/l L-glutamine and 25 mmol/l HEPES buffer (Life Technologies) supplemented with 10% heat-inactivated pooled human AB^+ serum (hereafter referred to as medium) were incubated in flat-bottom, 96-well plates (Costar, Cambridge, MA) with or without addition of Abs (PBMC: anti-CD3, clone 1XE, CLB, Amsterdam, The Netherlands; final dilution, 1/20,000; $CD3^+$ T cells: anti-CD3, clone $SpvT_3b$, provided by Dr. H. Spits, DNAX Research Laboratories, Palo Alto, CA; final dilution, 1:125,000), with or without different concentrations of

cAMP analogues as described below. Preliminary experiments had shown that the 1XE clone was the most potent inducer of PBMC, and the $SpvT_3b$ clone was the most potent inducer of $CD3^+$ T cells proliferation (data not shown); to optimize the experimental conditions, different mAbs were used for PBMC and T cell stimulation. In the $CD3^+$ T cell cultures, the cell surface markers were cross-linked using monodispersed immunomagnetic beads coated with sheep anti-mouse IgG (Dynal) at a cell-to-bead ratio of 1:1. After 48 h, 1 μCi of [^{3}H]thymidine was added to cell cultures, and 16 h later cultures were harvested onto glass filter strips, using an automated multisampler harvester (Scatron, Suffolk, U.K.) and were subsequently analyzed by beta scintillation counting. cAMP analogues (8-(4-chlorophenylthio)cAMP (8-CPT-cAMP; Sigma, St. Louis, MO) and Sp- and Rp-8-Br-cAMPS (BioLog Life Science, Bremen, Germany)), when used, were added to cell cultures 30 min before anti-CD3 stimulation. In some experiments different concentrations of human IL-2 (2,000 U/μg; Boehringer Mannheim, Mannheim, Germany) were added to cell cultures 30 min before stimulation.

Bromo-deoxy-uridine (BrdUrd) incorporation as determined by flow cytometry

Determination of BrdUrd incorporation in lymphocytes was performed as described previously (24) with some modifications. Briefly, negatively selected $CD3^+$ T cells were cultured in 24-well plates (Costar; 10^6 cells/ml, 1 ml/well) precoated with anti-CD3 Ab (clone $SpvT_3b$; final dilution, 1/1500) or without coat (unstimulated), with or without preincubation with different concentrations of 8-Br-cAMPS. After 48 h, BrdUrd (Sigma; final concentration, 30 μg/ml) was added to cell cultures. Sixteen hours later, the cells were washed once in PBS; resuspended in PBS with 5% mouse serum (Sigma), 5% human Ig (Octagam, Octapharma, Vienna, Austria), 2% BSA, and 0.1% sodium azide; and stained for membrane Ags by phycoerythrin-conjugated Abs (CD4, clone SK 3; CD8, clone SK 1; Becton Dickinson, San Jose, CA) for 30 min at 4°C. Thereafter, cells were washed in staining buffer (PBS with 1% FBS; Life Technologies), fixed in 1% paraformaldehyde (Sigma) and PBS with 0.01% Tween-20 (Sigma) overnight at 4°C, washed in PBS, and incubated in RPMI with 50 Kunitz units/ml DNase-1 (Sigma) at 37°C for 45 min. Cells were then incubated in 150 μl of PBS, with 10% BSA and 0.5% Tween-20, and 20 μl of FITC-conjugated anti-BrdUrd (Becton Dickinson) at 20°C for 45 min; washed twice; and finally resuspended in PBS. Samples were analyzed using a FACScan (Becton Dickinson) with CellQuest software (Becton Dickinson). List mode files were collected from 10,000 cells from each sample.

Determination of IL-2 levels

For determination of IL-2 levels, negatively selected $CD3^+$ T cells (10^6/ml, 200 μl/well) were cultured in medium alone or were stimulated with anti-CD3 Abs (clone $SpvT_3b$; final dilution 1/125,000) with or without preincubation with different concentrations of Rp-8-Br-cAMPS. The anti-CD3 Abs were cross-linked with immunomagnetic beads as described above. After 20 h of culture, cell-free supernatants were harvested and stored at -80°C until analysis. IL-2 levels in supernatants were determined by ELISA (R&D Systems, Minneapolis, MN).

cAMP determination

Extraction of cAMP and analyses of intracellular cAMP contents by RIA (Amersham, Aylesbury, U.K.)

in negatively selected $CD3^+$ T cells were performed as previously described (25). Basal levels of cAMP were stable at 4°C in both PBMC suspensions and negatively selected $CD3^+$ T cells for >120 min, i.e., the time required for isolation of $CD3^+$ T cells (data not shown).

Miscellaneous

The numbers of $CD4^+$, $CD8^+$, and $CD19^+$ lymphocytes in peripheral blood were determined by immunomagnetic quantification (22). Serum levels of neopterin were measured by RIA (IMMU test Neopterin, Henning Berlin, Berlin, Germany).

Statistical analysis

For comparison of two groups of individuals, the Mann-Whitney *U* test (two-tailed) was used. For comparison of parameters within the same individuals, the Wilcoxon signed rank test for paired data (two-tailed) was used. Coefficients of correlation were calculated by the Spearman rank test. Curve-fit analyses were performed using Sigma plot (Jandel, Erkrath, Germany). Data are given as medians and 25th to 75th percentiles if not otherwise stated. The p values are two-sided and are considered significant when <0.05.

Results

Top
Abstract
Introduction
Materials and Methods
Results
Discussion
References

Effect of cAMP antagonist on PBMC proliferation

To address the possible role of the cAMP/PKAI system in the impaired T cell function in CVI, we first examined whether a sulfur-substituted cAMP analogue (Rp-8-Br-cAMPS), working as a full antagonist for PKAI (26), could improve anti-CD3-stimulated proliferation of PBMC in 20 consecutively recruited CVI patients and 15 healthy controls. Confirming previous results (22, 27), stimulated lymphocyte proliferation was significantly impaired in this CVI population compared with that in control subjects (14,980 (9,100–21,340) cpm vs 63,340 (49,100–84,300) cpm; $p < 0.001$; CVI patients and controls, respectively). Furthermore, while antagonist did not significantly alter the proliferation of lymphocytes obtained from normal blood donors, Rp-8-Br-cAMPS induced a significant and concentration-dependent improvement of anti-CD3-stimulated proliferation in the CVI group (fold increase with maximal Rp-8-Br-cAMPS concentration (1000 μM), 1.63 (1.39–2.37) vs 1.07 (1.05–1.12); $p < 0.001$; CVI patients and controls, respectively). However, single patient data from the CVI group revealed heterogeneity. Whereas a >100% increase in anti-CD3-induced lymphocyte proliferation was found in seven of the CVI patients, five of the patients had a <40% increase in proliferation when the cAMP antagonist was added to cells in vitro. Of note, the patients with the most marked increase in lymphocyte proliferation after stimulation with the cAMP antagonist were those with the most severely depressed proliferative response after anti-CD3 stimulation ($r = -0.85$; $p < 0.001$).

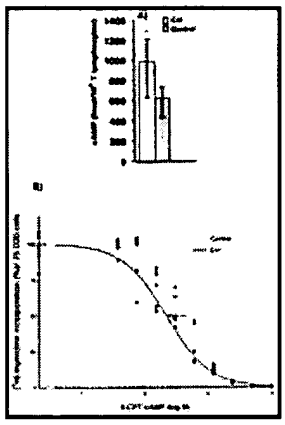
Bacterial products and endotoxins may influence the proliferation of PBMC, but CVI patients with chronic rhinosinusitis and bronchiectasis did not differ from other patients with respect to either

proliferation or the effect of cAMP antagonist (data not shown). Furthermore, when examining the effect of cAMP antagonist on anti-CD3-stimulated proliferation of PBMC from three patients with X-linked agammaglobulinemia (XLA), a subgroup of primary hypogammaglobulinemia not characterized by T cell deficiency (11, 12), all had a <20% increase in lymphocyte proliferation after addition of cAMP antagonist, although the duration of replacement therapy and clinical symptoms was longer, and the occurrence of chronic infectious complications increased in these XLA patients compared with those in the CVI group (data not shown).

Effects of cAMP agonist and antagonist in purified T cells

CVI patients represent a heterogeneous group of patients, and T cell deficiency is a significant feature in only a subgroup of patients. When further examining the possible role of cAMP/PKAI in the T cell deficiency in CVI, we therefore in subsequent experiments studied CVI patients characterized by markedly and persistently impaired T cell function based on the following criteria: 1) anti-CD3-stimulated lymphocyte proliferation <15% of median levels in healthy controls, and 2) this impaired T cell function should have been confirmed at least three times during the last 5 yr. Seven of the CVI patients fulfilled these criteria, and these patients were compared with eight of the healthy controls. These CVI patients with T cell deficiency were not different from the other CVI patients with respect to Ig dosage, duration of Ig replacement therapy, duration of symptoms, or occurrence of infectious complications. However, they had significantly higher serum neopterin levels as a marker of monocyte hyperactivity (22) than CVI patients without strictly defined T cell deficiency (38.6 26, 3–65.8) vs 19.3 (10.8–28.7) nmol/l; $p < 0.01$). Furthermore, while all CVI patients with T cell deficiency had splenomegaly, this was found in only three of the other 13 CVI patients.

When analyzing cAMP levels in negatively selected purified T lymphocytes from CVI patients with T cell deficiency and controls, we found significantly higher cAMP levels in the CVI group (Fig. 1*A*). The sensitivity to cAMP-dependent inhibition of T cell proliferation was also increased in CVI, showing the positive cooperative effect of endogenous cAMP levels (Fig. 1*B*), and this may be a more stable and reliable marker of endogenous cAMP levels than the actual cAMP level as determined by RIA. The results presented in Table II show such an effect of 8-CPT-cAMP on cell proliferation in all CVI patients with impaired T cell function compared with the effect in the eight control subjects. This significant increase in sensitivity to inhibition of cell proliferation by exogenously added 8-CPT-cAMP in the CVI group was reflected in a marked decrease in IC_{50} values in these patients, primarily due to a change in the slope for the inhibition curve (Hill coefficient; Table II and Fig. 1*B*).



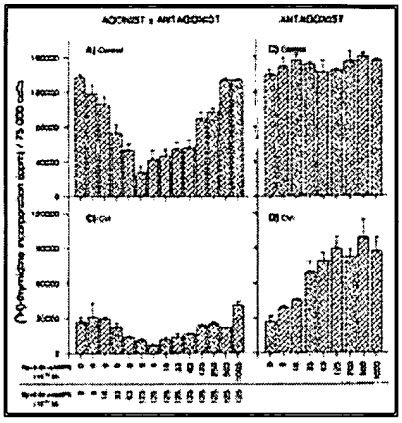
View larger version (16K):
[in this window]
[in a new window]

FIGURE 1. Endogenous cAMP levels in T cells from seven CVI patients with impaired T cell function and eight healthy controls. *A*, cAMP levels in negatively selected T cells. Data are given as medians. Error bars represent 25th-75th percentiles. $_{*}p < 0.05$ vs controls. *B*, The effect of increasing concentrations of the cAMP agonist 8-CPT-cAMP on anti-CD3-stimulated T cell proliferation in one representative CVI patient (solid circles) and one healthy control (open circles) are shown. Maximal proliferation was normalized to 100%. Curve-fit analyses were performed using Sigma plot, and IC_{50} values were calculated, demonstrating markedly decreased values for T cells from the CVI patient compared with the control (2.26 vs 4.66 μM). For statistics between the CVI group and controls, see Table II. Note the leftward shift of IC_{50} (arrow) and the altered curve slope (Hill coefficient).

View this table:
[in this window]
[in a new window]

Table II. Effect of cAMP antagonist and agonist on anti-CD3-stimulated T cell proliferation in seven CVI patients with impaired T cell function and eight healthy controls[1]

To further address the specificity of the inhibition of anti-CD3-stimulated T cell proliferation we used a cAMP agonist (Sp-8-Br-cAMPS) and its complementary PKAI-selective antagonist (Rp-8-Br-cAMPS). In healthy controls the inhibitory effect of the cAMP agonist was completely reversed by its complementary antagonist (Fig. 2*A*), but the antagonist alone did not further enhance T cell proliferation (Fig. 2*B* and Table II). In contrast, we found a concentration-dependent increase in anti-CD3-stimulated proliferation in CVI patients (>100% increase in three patients and reaching levels within the normal range in two patients) when Rp-8-Br-cAMPS was added to cell cultures (Fig. 2, *C* and *D*, and Table II). Thus, it seems that in CVI patients, T cells with impaired anti-CD3-stimulated proliferation are characterized by chronically elevated endogenous cAMP levels, and treatment with a selective PKAI antagonist markedly improves anti-CD3-stimulated proliferation in these cells, reaching proliferation levels comparable to control values (~25 and 75% of levels in healthy controls, with and without Rp-8-Br-cAMPS, respectively).



View larger version (69K):
[in this window]
[in a new window]

FIGURE 2. Modulation of T cell proliferation by cAMP agonist and antagonist in CVI patients with impaired T cell function and in healthy controls. Inhibition of anti-CD3-stimulated proliferation of T cells by the cAMP agonist (Sp-8-Br-cAMS) and reversal of inhibition by its complementary PKA type I-selective antagonist (Rp-8-Br-cAMPS) are shown in one healthy control (*A*) and one representative CVI patient (*C*). The effect of increasing concentrations of Rp-8-Br-cAMPS on anti-CD3-stimulated T lymphocyte proliferation was also examined separately, and the results from the same control and CVI patient shown in *A* and *C* are given in *B* and *D*, respectively. Data are given as the mean value for triplicate determinations ± SD. For statistics between the CVI group and controls, see Table II.

Effects of cAMP antagonist on proliferation of $CD4^+$ and $CD8^+$ T cells

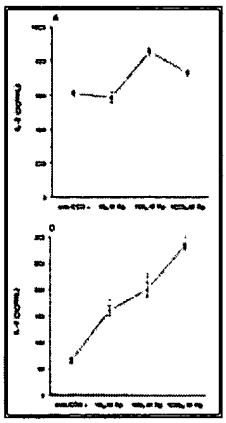
We next, by flow cytometric analysis of BrdUrd incorporation, examined anti-CD3-stimulated T cells DNA synthesis in the presence and the absence of Rp-8-Br-cAMPS in subsets of $CD4^+$ and $CD8^+$ T cells from the seven CVI patients with impaired T cell function (see above) and the seven controls. In CVI patients there was a significant increase in the percentage of $BrdUrd^+$ $CD4^+$ T cells when cAMP antagonist was added to cell culture (61.3% 33.6–86.0%) vs 80.0% (47.0–95.8%), without and with antagonist, respectively; $p < 0.05$). In most patients the maximal increase was found at the highest concentration of Rp-8-Br-cAMPS (1000 μM). No effect of Rp-8-Br-cAMPS on DNA synthesis was seen in $CD4^+$ T cells from healthy controls (data not shown). For $CD8^+$ T cells there was no significant increase in the percentage of $BrdUrd^+$ cells after addition of cAMP antagonist in either CVI patients or controls, although a modest increase was seen in three CVI patients (data not shown).

Effect of cAMP antagonist on IL-2 levels in T cell supernatants

IL-2 plays a pivotal role in the growth and function of T cells (28), and decreased IL-2 production from these cells may play an important role in the immunopathogenesis of CVI (29, 30). cAMP decreases IL-2 production in T cells (31), and to further elucidate the mechanism(s) of cAMP-induced inhibition of T cell proliferation in CVI, we examined the effect of Rp-8-Br-cAMPS on IL-2 levels in supernatants from anti-CD3-stimulated T lymphocytes in the seven CVI patients with impaired T cell function and the eight controls. Compared with control subjects, T cells from CVI patients released significantly lower IL-2 levels into supernatants (Table III), and we found a marked and concentration-dependent increase in IL-2 levels in the presence of cAMP antagonist (Table III and Fig. 3). The effect of Rp-8-Br-cAMPS on IL-2 levels of CVI T cells was largely similar to that on proliferation. However, despite the dramatic increase in IL-2 levels after addition of Rp-8-Br-cAMPS to cell cultures in CVI patients, the IL-2 level was still markedly lower than that in control subject (Table III). Thus, in this subgroup of CVI patients, T cell proliferation is normalized to a greater extent than IL-2 secretion by addition of cAMP antagonist to cells in vitro.

View this table: [in this window] [in a new window]

Table III. Effect of cAMP antagonist (Rp-8-Br-cAMPS) on IL-2 levels in supernatants from anti-CD3- stimulated T cells seven CVI patients with impaired T cell function and eight healthy controls[1]

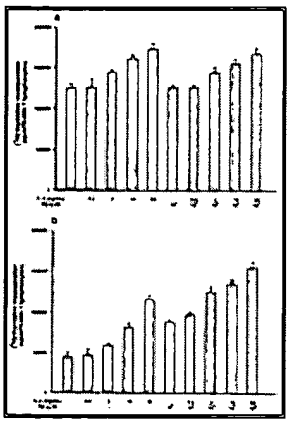


View larger version (10K): [in this window] [in a new window]

FIGURE 3. Effect of cAMP antagonist on the release of IL-2 from T cells in CVI patients with impaired T cell function and in healthy controls. IL-2 levels in supernatants from anti-CD3-stimulated T cells after 20 h of culture with or without the addition of different concentrations of the selective PKA type I antagonist Rp-8-Br-cAMPS (Rp) are shown. *A*, Healthy control. *B*, Representative CVI patient. Data are given as the mean value for triplicate determinations ± SD. For statistics between the CVI group and controls, see Table III. $_{*}$, $p < 0.01$ vs IL-2 level without antagonist. $_{**}$, $p < 0.001$ vs IL-2 level without antagonist.

Effect of exogenously added IL-2 in combination with cAMP antagonist on anti-CD3-induced T cell proliferation

To further examine the role of IL-2 in the enhancement of T cell proliferation by addition of cAMP antagonist, we examined the effect of exogenously added IL-2, either alone or in combination with Rp-8-Br-cAMPS, on anti-CD3-stimulated T cell proliferation in the seven CVI patients with T cell deficiency and the eight controls. After addition of IL-2 to cell culture there was a marked increase in proliferation in both CVI patients and controls (Fig. 4). However, at IL-2 concentrations comparable to the achieved increase in IL-2 levels after addition of cAMP antagonist (~ 0.20 ng/ml; Fig. 3*B*), no significant effect was seen on proliferation in either CVI patients or controls (Fig. 4). In fact, the enhancing effect of cAMP antagonist in CVI patients was comparable to the effect of 10 ng/ml IL-2, i.e., a 50-fold higher concentration (Fig. 4). Furthermore, in CVI patients the enhancing effects of Rp-8-Br-cAMPS and IL-2 on T lymphocyte proliferation were additive at all concentrations tested (Fig. 4).



View larger version (42K):
[in this window]
[in a new window]

FIGURE 4. Modulation of T cell proliferation by IL-2 and cAMP antagonist in CVI patients with impaired T cell function and in healthy controls. The effect of increasing concentrations of IL-2 (2 U/ng) with or without 1000 μM of Rp-8-Br-cAMPS (Rp) on anti-CD3-stimulated T cell proliferation are shown for one healthy control (*A*) and one representative CVI patient (*B*). Data are given as the mean value for triplicate determinations ± SD.

Discussion

- Top
- Abstract
- Introduction
- Materials and Methods
- Results
- Discussion
- References

The present study demonstrates for the first time increased endogenous cAMP levels in T cells from CVI patients with impaired T cell function and, even more importantly, that a selective inhibition of PKAI could markedly improve or in some cases even fully restore anti-CD3-induced T cell proliferation in this subgroup of CVI patients. These findings indicate a possible intracellular mechanism for the T cell defect in CVI and suggest that the cAMP/PKAI system may be a potential target for immunomodulating therapy in these patients.

Some reports have previously studied the intracellular basis for the T cell defect in CVI. Eibl and co-workers found impaired proliferation, decreased formation of inositol 1,4,5-triphosphate (Ins(1, 4, 5)P_3), and reduced capacity to mount an increase in intracellular Ca^{2+} after TCR stimulation in T cells from CVI patients (17, 18). However, the impaired Ins(1, 4, 5)P_3 formation was not absolute, as indicated by a normal response after direct G protein activation by aluminum-fluoride (18). Furthermore, stimulation with phorbol esters and calcium ionophore resulting in direct PKC activation and mobilization of Ca^{2+} from intracellular stores does not seem to be deficient in CVI (16, 17, 18, 29). Also, the CD28 and IL-2 receptor signaling pathways appear to be functional in T cells from CVI patients, but neither IL-2 nor costimulation via CD28 could correct the T cell dysfunction after TCR/CD3 stimulation (18, 32, 33). Together, these studies suggest that the T cell defect in a subgroup of CVI patients is caused at least in part by an early defect in T cell activation after triggering of the TCR/CD3 complex before the generation of Ins(1, 4, 5)P_3 and PKC activation.

We have suggested that cAMP leading to activation of PKAI is an important inhibitor of normal T cell proliferation after Ag stimulation (3, 6, 20). Although cAMP agonists may inhibit T cell function at more than one site (31, 34), several lines of evidence indicate that PKAI is an early inhibitor of T cell

activation after TCR/CD3 stimulation. PKAI activation inhibits TCR/CD3-dependent hydrolysis of inositol phospholipids to Ins(1, 4, 5)P_3 and diacylglycerol as well as early tyrosine phosphorylation (20). Furthermore, T cell activation by direct PKC stimulation by phorbol esters seems to be insensitive to inhibition by cAMP/PKAI (20). Finally, PKAI colocalizes to the TCR/CD3 complex upon activation and capping, and may release kinase activity that, through phosphorylation, might uncouple the TCR/CD3 complex from the intracellular signaling pathway (6). Thus, although our findings of increased activation of the cAMP/PKAI system in T cells from CVI patients may not necessarily be related to the basic molecular defect(s) that leads to CVI, these findings strongly suggest a defect in the early phase of T cell activation after TCR/CD3 triggering in these patients and, more importantly, that increased cAMP/PKAI activation may contribute to the molecular basis for this defect.

CVI represents a heterogeneous group of patients, both clinically and immunologically (35). As for the T cell defect, both subgroups with predominantly $CD4^+$ and predominantly $CD8^+$ T cell involvement have been reported (29, 36). Although the CVI patients in the present study had decreased $CD4^+$ and tended to have increased $CD8^+$ T cell counts compared with controls, our flow cytometric data analyzing the effect of cAMP antagonist on these T cell subsets separately, suggest that the enhanced PKAI activity in T cells from CVI patients with T cell deficiency does not merely reflect altered distribution of $CD4^+$ and $CD8^+$ subsets. In fact, our findings suggest that increased PKAI activity in this subgroup of CVI patients mostly affects $CD4^+$ lymphocytes, which in these patients represent a smaller proportion of T cells than in controls.

Our findings of impaired IL-2 production from T cells in CVI in this study confirm previous reports (17, 18, 29), and the results suggest that increased PKAI activity may contribute to this impairment. However, the induction of enhanced T cell proliferation in CVI, mediated by cAMP antagonist, appears not to be dependent on increased IL-2 release. Exogenously added IL-2 had no effect on T cell proliferation at concentrations comparable to the achieved increase in IL-2 levels after addition of cAMP antagonist. Furthermore, the stimulatory effects of IL-2 and cAMP antagonist on TCR/CDR-stimulated T cell proliferation were additive at all concentrations tested and did not saturate each other. Thus, the effect of IL-2 and cAMP antagonist on T cell proliferation in CVI are distinct and possibly mediated by separate mechanisms. An additive effect between IL-2 and cAMP antagonist may be of particular interest also from a therapeutical point of view. Both in vivo and in vitro studies suggest that IL-2 may improve, but not fully correct, the T cell function in CVI (17, 18, 37), and it is tempting to hypothesize that the combination of IL-2 therapy and therapeutical interventions that down-regulate PKAI activity may be an interesting approach to immunomodulation in CVI.

Immunologic abnormalities in CVI might be secondary phenomena to infectious complications and therapy. However, we could not find any association between the presence of chronic bacterial complications and abnormalities in the cAMP/PKAI system in T cells from CVI patients. IgG may alter several immunologic functions both in vitro and in vivo (38), but in the present study blood samples were collected just before Ig substitution to minimize the effect of such therapy. Moreover, s.c. Ig administration, which was used by all CVI patients in the present study, in contrast to i.v. Ig therapy, does not affect lymphocyte and monocyte functions (39). Furthermore, among CVI patients there was no association between either Ig dosage or duration of Ig therapy and the magnitude of response to cAMP

antagonist. Finally, although the duration of replacement therapy and clinical symptoms was longer and the occurrence of chronic infectious complications increased in XLA patients compared with those in the CVI group, the effect of cAMP antagonist on lymphocyte proliferation in XLA patients was comparable to the effect in healthy controls.

We have previously demonstrated a persistent monocyte activation in a subgroup of CVI patients (22, 40), and monocyte products such as IL-1 and PGE_2, which appear to be elevated in CVI (P. Aukrust, F. Müller, and S. S. Frøland, unpublished observations), may increase cAMP levels in T cells (2, 31). In fact, CVI patients with T cell deficiency and enhanced PKAI activity had significantly higher serum neopterin levels, as a marker of monocyte hyperactivity (22), than other CVI patients. Furthermore, these T cell-deficient CVI patients had increased occurrence of splenomegaly, a finding that has previously been associated with monocyte hyperactivity and T cell deficiency in CVI (27, 41). Thus, there may well be a pathogenic link between monocyte hyperactivity and cAMP/PKAI-mediated T cell dysfunction in CVI, and studies examining this possibility are ongoing in our laboratory. Furthermore, retroviral peptides and other viral components have been found to increase cAMP levels in lymphocytes (42), and several authors have suggested that CVI may develop as a result of a chronic viral infection in genetically susceptible individuals (36, 43), possibly representing a slowly progressive, not stagnant, disorder of the immune system. Immunologic similarities to HIV infection (12, 13, 21, 27), as also demonstrated in the present study, i.e., abnormalities in the cAMP/PKAI system (44), may further support such an idea. Whatever the reasons, although several mechanisms may be involved in the pathogenesis of T cell deficiency in CVI, the demonstration of an up to 3.7-fold increase in T cell proliferation after addition of cAMP antagonist in CVI patients with impaired T cell function, clearly suggests that the increased PKAI activity may represent an important feature of T cells in these patients.

While cAMP is mitogenic in differentiated endocrine cells, in vitro studies suggest that the cAMP/PKAI system generally delivers an off signal for many functions in the immune system (2, 3, 4). The results of the present study in addition to recent reports demonstrating impaired cAMP/PKAI activity in T cells from patients with systemic lupus erythematosus and increased cAMP/PKAI activity in T cells from HIV-infected patients (9, 44) further support an important immunoregulatory role for the cAMP/PKAI system in vivo in human disorders. Further studies addressing the role of increased PKAI activation in the pathogenesis of T cell deficiency in CVI may be of great interest.

Acknowledgments

We thank Bodil Lunden, Lisbeth Wikeby, and Vigdis Bjerkeli for excellent technical assistance.

Footnotes

[1] This work was supported by the Norwegian Cancer Society, the Norwegian Research Council, the Novo Nordisk Foundation Committee, the Anders Jahres Foundation, the Medinnova Foundation, and Odd Kåre Rabben's Memorial Fund for AIDS Research.

[2] Address correspondence and reprint requests to Dr. Pål Aukrust, Section of Clinical Immunology and Infectious Diseases, Medical Department A, Rikshospitalet, N-0027, Oslo, Norway. E-mail address:

[3] Abbreviations used in this paper: PKA, protein kinase A; PKAI, PKA type I; CVI, common variable immunodeficiency; PKC, protein kinase C; 8-CPT-cAMP, 8-(4-chlorophenylthio)cAMP 3',5', Sp- and Rp-8-Br-cAMPs, Sp- and Rp-8-bromo-cAMP-phosphorothioate; BrdUrd, bromodeoxyuridine; XLA, X-linked agammaglobulinemia; $Ins(1,4,5)P_3$, inositol 1,4,5-triphosphate.

Received for publication July 6, 1998. Accepted for publication September 25, 1998.

References

Top
Abstract
Introduction
Materials and Methods
Results
Discussion
References

1. Muraguchi, A., K. Miyazaki, J. H. Kehrl, A. S. Fauci. 1984. Inhibition of B cell activation by diterpine forskolin: interference with B cell growth factor-induced G1 to S transition of the B cell cycle. *J. Immunol. 133:1283.*[Abstract]
2. Kammer, G. M.. 1988. The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. *Immunol. Today 9:222.*[Medline]
3. Skålhegg, B. S., B. F. Landmark, S. O. Døskeland, V. Hansson, T. Lea, T. Jahnsen. 1992. Cyclic AMP-dependent protein kinase type 1 mediate the inhibitory effects of 3',5'-cyclic adenosine monophosphate on cell replication in human T lymphocytes. *J. Biol. Chem. 256:15707.*
4. Scott, J. D.. 1991. Cyclic nucleotide-dependent protein kinases. *Pharmacol. Ther. 50:123.*[Medline]
5. Francis, S. H., J. D. Corbin. 1994. Structure and function of cyclic nucleotide-dependent protein kinases. *Annu. Rev. Physiol. 56:237.*[Medline]
6. Skålhegg, B. S., K. Tasken, V. Hansson, H. S. Huitfeldt, T. Jahnsen, T. Lea. 1994. Location of cAMP-dependent protein kinase type 1 with the TCR/CD3 complex. *Science 263:84.*[Medline]
7. Ledbetter, J. A., M. Parson, P. J. Martin, J. A. Hansen, P. S. Rabinovitch, C. H. June. 1986. Antibody binding to CD5 (Tp67) and Tp44 T cell surface molecules: effect on cyclic nucleotides, cytoplasmatic free calcium, and cAMP-mediated suppression. *J. Immunol. 137:3299.*[Abstract]
8. Kammer, G. M., C. A. Boehm, S. A. Rudolph, L. A. Schultz. 1988. Mobility of the human T lymphocyte surface molecules CD3, CD4, and CD8: regulation by cAMP-dependent pathway. *Proc. Natl. Acad. Sci. USA 85:792.*[Medline]
9. Kammer, G. M., I. U. Khan, C. J. Malemud. 1994. Deficient type 1 protein kinase A isoenzyme activity in systemic lupus erythematosus. *J. Clin. Invest. 94:422.*[Medline]
10. Spickett, G. P., S. A. Misbah, H. M. Chapel. 1991. Primary antibody deficiency in adults. *Lancet 337:281.*[Medline]
11. 1995. Primary immunodeficiency diseases. *Clin. Exp. Immunol. 97:(Suppl. 1):1.*
12. Spickett, G. P., A. D. B. Webster, J. Farrant. 1990. Cellular abnormalities in common variable immunodeficiency. *Immunodefic. Rev. 2:199.*[Medline]
13. Jaffe, J. S., E. Eisenstein, M. C. Sneller, W. Strober. 1993. T-cell abnormalities in common variable immunodeficiency. *Pediatr. Res. 33:(Suppl.):S24.*[Medline]
14. Mayer, L. S., S. M. Fu, C. Cunningham-Rundles, H. G. Kunkel. 1984. Polyclonal immunoglobulin secretion in patients with common variable immunodeficiency using monoclonal B cell differentiation factor. *J. Clin. Invest. 74:2115.*[Medline]
15. Nonoyama, S., M. Farrington, H. Ishida, M. Howard, H. D. Ochs. 1993. Activated B cells from

patients with common variable immunodeficiency proliferate and synthesize immunoglobulin. *J. Clin. Invest. 92:1282.*[Medline]

16. Fiedler, W., K. W. Sykora, K. Welte, J. E. Kolitz, C. Cunningham-Rundles, K. Holloway, G. A. Miller, L. Souza, R. Mertelsmann. 1987. T-cell activation defect in common variable immunodeficiency: restoration by phorbol myristate acetate (PMA) or allogenic macrophages. *Clin. Immunol. Immunopathol. 44:206.*[Medline]
17. Fischer, M. B., I. Hauber, H. Eggenbauer, V. Thon, E. Vogel, E. Schaffer, J. Lokaj, J. Litzman, H. M. Wolf, J. W. Mannhalter, M. M. Eibl. 1994. A defect in the early phase of T-cell receptor-mediated T-cell activation in patients with common variable immunodeficiency. *Blood 84:4234.*[Abstract]
18. Fischer, M. B., H. M. Wolf, I. Hauber, H. Eggenbauer, V. Thon, M. Sasgary, M. M. Eibl. 1996. Activation via the antigen receptor is impaired in T cells, but not in B cells from patients with common variable immunodeficiency. *Eur. J. Immunol. 26:231.*[Medline]
19. Laxminarayana, D., A. Berrada, G. M. Krammer. 1993. Early events of human T lymphocyte activation are associated with type I protein kinase A activity. *J. Clin. Invest. 92:2207.*[Medline]
20. Skålhegg, B. S., A. M. Rasmussen, K. Taskén, V. Hansson, T. Jahnsen, T. Lea. 1994. Cyclic AMP sensitive signaling by the CD28 marker requires concomitant stimulation by the T-cell antigen receptor (TCR/CD3) complex. *Scand. J. Immunol. 40:201.*[Medline]
21. Aukrust, P., F. Müller, S. S. Frøland. 1994. Elevated serum levels of interleukin-4 and interleukin-6 in patients with common variable immunodeficiency (CVI) are associated with chronic immune activation and low numbers of $CD4^+$ lymphocytes. *Clin. Immunol. Immunopathol. 770:217.*
22. Aukrust, P., S. S. Frøland, F. Müller. 1992. Raised serum neopterin levels in patients with primary hypogammaglobulinaemia; correlation to other immunological parameters and to clinical and histological features. *Clin. Exp. Immunol. 89:211.*[Medline]
23. Müller, F., H. Rollag, G. Gaudernack, S. S. Frøland. 1990. Impaired in vitro survival of monocytes from patients with HIV infection. *Clin. Exp. Immunol. 81:25.*[Medline]
24. Esin, S., G. Batoni, H. Källenius, H. Gaines, M. Campa, S. B. Svenson, R. Andersson, H. Wigzell. 1996. Proliferation of distinct human T cell subsets in response to live, killed or soluble extracts of *Mycobacterium tuberculosis* or *Mycobacterium avium*. *Clin. Exp. Immunol. 104:419.*[Medline]
25. Tasken, K., K. B. Andersson, B. S. Skålhegg, K. A. Tasken, V. Hansson, T. Jahnsen, H. K. Blomhoff. 1993. Reciprocal regulation of mRNA and protein for subunits of cAMP-dependent protein kinase (RIα and Cα) by cAMP in a neoplastic B cell line (Reh). *J. Biol. Chem. 268:23483.*[Abstract/Full Text]
26. Gjertsen, B. T., G. Mellgren, A. Otten, E. Maronde, H. G. Genieser, B. Jastorf, O. K. Vintermyr, G. S. McKnight, S. O. Døskeland. 1995. Novel (*Rp*)-cAMPS analogs as tools for inhibition of cAMP-kinase in cell culture. *J. Biol. Chem. 270:20599.*[Abstract/Full Text]
27. Aukrust, P., A. M. Svardal, F. Müller, B. Lunden, R. K. Berge, S. S. Frøland. 1995. Decreased levels of total and reduced glutathione in $CD4^+$ lymphocytes in common variable immunodeficiency are associated with activation of the tumor necrosis factor α system: possible immunopathogenic role of oxidative stress. *Blood 86:1383.*[Abstract]
28. Waldman, T. A.. 1991. The interleukin-2 receptor. *J. Biol. Chem. 266:2681.*[Full Text]
29. Eisenstein, E. M., J. S. Jaffe, W. Strober. 1993. Reduced interleukin-2 (IL-2) production in common variable immunodeficiency is due to a primary abnormality of $CD4^+$ T cell differentiation. *J. Clin. Immunol. 13:247.*[Medline]
30. Cunningham-Rundles, C., K. Kazbay, J. Hassett, Z. Zhou, L. Mayer. 1994. Enhanced humoral immunity in common variable immunodeficiency after long-term treatment with polyethylene glycol-conjugated interleukin-2. *N. Engl. J. Med. 331:918.*[Full Text]
31. Anastassiou, E. D., F. Paliogianni, J. P. Balow, H. Yamada, D. T. Boumpas. 1992. Prostaglandin

E_2 and other cyclic AMP-elevating agents modulate IL-2 and IL-2Rα gene expression at multiple levels. *J. Immunol. 148:2845.*[Abstract]

32. Fischer, M. B., H. M. Wolf, H. Eggenbauer, V. Thon, E. Vogel, J. Lokaj, J. Litzman, J. W. Mannhalter, M. M. Eibl. 1994. The costimulatory signal CD28 is fully functional but cannot correct the impaired antigen response in T cells of patients with common variable immunodeficiency. *Clin. Exp. Immunol. 95:209.*[Medline]
33. North, M. E., A. N. Akbar, N. Borthwick, K. Sagawa, M. Funauchi, A. D. B. Webster, J. Farrant. 1994. Co-stimulation with anti-CD28 (Kolt-2) enhances DNA synthesis by defective T cells in common variable immunodeficiency. *Clin. Exp. Immunol. 95:204.*[Medline]
34. Paliogianni, F., R. L. Kincaid, D. T. Boumpas. 1993. Prostaglandin E_2 and other cyclic AMP elevating agents inhibit interleukin 2 gene transcription by counteracting calcineurin-dependent pathways. *J. Exp. Med. 178:1813.*[Abstract]
35. Spickett, G. P., J. Farrant, M. North, J.-G. Zhang, L. Morgan, A. D. B. Webster. 1997. Common variable immunodeficiency: how many diseases?. *Immunol. Today 18:325.*[Medline]
36. Jaffe, J. S., W. Strober, M. C. Sneller. 1993. Functional abnormalities of $CD8^+$ T cells define a unique subset of patients with common variable immunodeficiency. *Blood 82:192.*[Abstract]
37. Rump, J. A., A. Jahreis, M. Schlesier, S. Stecher, P. H. Peter. 1997. A double blind, placebo-controlled, crossover therapy study with natural human IL-2 (nhuIL-2) in combination with regular intravenous gammaglobulin (IVIG) infusions in 10 patients with common variable immunodeficiency (CVID). *Clin. Exp. Immunol. 110:167.*[Medline]
38. Andersson, U., L. Björk, U. Skansén-Sapir, J. Andersson. 1994. Pooled human IgG modulates cytokine production in lymphocytes and monocytes. *Immunol. Rev. 139:325.*
39. Achiron, A., R. Margalit, R. Herschkoviz, D. Markovits, T. Reshef, T. E. Melamed, J. R. Cohen, O. Lider. 1994. Intravenous immunoglobulin treatment of experimental T-cell mediated autoimmune disease. *J. Clin. Invest. 193:600.*
40. Aukrust, P., E. Lien, A. K. Kristoffersen, F. Müller, C. J. Haug, T. Espevik, S. S. Frøland. 1996. Persistent activation of the tumor necrosis factor system in a subgroup of patients with common variable immunodeficiency: possible immunological and clinical consequences. *Blood 87:674.*[Abstract]
41. Mulligham, C. G., G. C. Fanning, H. M. Chapel, K. I. Welsh. 1997. TNF and lymphotoxin-α polymorphisms associated with common variable immunodeficiency: role in the pathogenesis of granulomatous disease. *J. Immunol. 159:6236.*[Abstract]
42. Haraguchi, S., R. A. Good, N. K. Day. 1995. Immunosuppressive retroviral peptide: cAMP and cytokine patterns. *Immunol. Today 16:595.*[Medline]
43. Spickett, G. P., M. Millrain, R. Beattie, M. North, J. Griffiths, S. Patterson, A. D. B. Webster. 1988. Role of retroviruses in acquired hypogammaglobulinaemia. *Clin. Exp. Immunol. 74:177.*[Medline]
44. Aandahl, E. M., P. Aukrust, B. S. Skålhegg, F. Müller, S. S. Frøland, V. Hansson, K. Taskén. 1998. Protein kinase type I antagonist restores immune responses of T-cells from HIV-infected patients. *FASEB J. 12:855.*[Abstract/Full Text]

This article has been cited by other articles:

- Abstract of this Article
- **Reprint (PDF) Version of this Article**
- Similar articles found in:
  The JI
  PubMed
- PubMed Citation
- This Article has been cited by:
- Search Medline for articles by:
  Aukrust, P. || Müller, F.
- Alert me when:
  new articles cite this article
- Download to Citation Manager

- Dahle, M. K., Knutsen, H. K., Tasken, K. A., Pilz, R., Tasken, K. (2001). Cyclic AMP regulates expression of the RI{alpha} subunit of cAMP-dependent protein kinase through an alternatively spliced 5' UTR. *Eur J Biochem* 268: 5920-5929 [Abstract] [Full Text]
- Vang, T., Torgersen, K. M., Sundvold, V., Saxena, M., Levy, F. O., Skålhegg, B. S., Hansson, V., Mustelin, T., Taskén, K. (2001). Activation of the COOH-terminal Src kinase (Csk) by cAMP-dependent Protein Kinase Inhibits Signaling through the T Cell Receptor. *J. Exp. Med.* 193: 497-508 [Abstract] [Full Text]

HOME HELP FEEDBACK SUBSCRIPTIONS ARCHIVE SEARCH TABLE OF CONTENTS